AF

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 March 2001 (15.03.2001)

PCT

(10) International Publication Number
WO 01/017559 A1

(51) International Patent Classification[7]: A61K 39/395, 38/17, A61P 35/00, 37/00

(21) International Application Number: PCT/US00/24556

(22) International Filing Date:
7 September 2000 (07.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/152,915	8 September 1999 (08.09.1999)	US
60/172,877	20 December 1999 (20.12.1999)	US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:

US	60/152,915 (CON)
Filed on	8 September 1999 (08.09.1999)
US	60/172,877 (CON)
Filed on	20 December 1999 (20.12.1999)

(71) Applicant *(for all designated States except US)*: **IMMUNEX CORPORATION** [US/US]; 51 University Street, Seattle, WA 98101-2936 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SPRIGGS, Melanie, Kay** [US/US]; 2256 12th Avenue West, Seattle, WA 98119 (US). **COMEAU, Michael, Rene** [US/US]; 8349 Jones Avenue NW, Seattle, WA 98117 (US).

(74) Agent: **DAVIES, Tracey, B.**; Vinson & Elkins L.L.P., 2300 First City Tower, 1001 Fannin, Houston, TX 77002-6760 (US).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(48) Date of publication of this corrected version:
21 November 2002

(15) Information about Correction:
see PCT Gazette No. 47/2002 of 21 November 2002, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: SEMAPHORIN MODULATION OF IMMUNE CELL MIGRATION

(57) Abstract: The present invention is directed to compositions and methods for use in immunotherapeutic treatment of immune cell migration-mediated diseases or conditions, including the following: auto-immune; hypersensitivity or neoplastic diseases or conditions such as cancers; and food intolerance. Also encompassed by the invention are methods and compositions for use in promoting or suppressing angiogenesis. The compositions and methods disclosed involve the use of semaphorin polypeptides and semphorin receptor polypeptides, which function to influence immune cell migration.

WO 01/017559 A1

SEMAPHORIN MODULATION OF IMMUNE CELL MIGRATION

This application claims priority to United States provisional patent application serial number 60/152,915 filed September 8, 1999, now abandoned; and United States provisional patent application serial number 60/172,877, filed December 20, 1999, now abandoned.

I. FIELD OF THE INVENTION

The present invention is directed to compositions and methods useful in immunotherapeutics. The agents used in these compositions and methods are semaphorin polypeptides, including semaphorin receptor polypeptides, that, through receptor-mediated activity, function to direct migration of immune cells.

II. BACKGROUND OF THE INVENTION

The semaphorin gene family includes, to date, over twenty molecules that encode related transmembrane and secreted glycoproteins known to be neurologic regulators. Semaphorins are generally well conserved in their extracellular domains, which are typically about 500 amino acids in length, and are best known in the field of neurobiology as chemorepulsive molecules that work with chemoattractants to direct the migration of developing axons (Tessier-Lavigne, M and Goodman C.S., 1996). Semaphorins have been studied largely for their role in neuronal growth cone guidance. For example, the secreted semaphorins known as collapsin-1 and Drosophila semaphorin II are selectively involved in repulsive growth cone guidance during development. Flies having mutated to display reduced activity of semaphorin II genes exhibit abnormal behavior characteristics.

Mammalian (human, rat, and mouse) semaphorin genes have also been identified, based upon their similarity to insect semaphorins. Functional studies of these semaphorins suggest that embryonic and adult neurons require a semaphorin to establish workable connections. Significantly, the fast response time of growth cone cultures to appropriate semaphorins suggests that semaphorin signaling involves a receptor-mediated signal transduction mechanism. Accordingly, it is speculated that semaphorin ligands secreted into the extracellular milieu signal through receptor bearing cells in a localized and systemic fashion.

In addition to insect and mammalian semaphorin genes, viral semaphorin genes have also been identified. One viral semaphorin gene is found in several strains of poxvirus including both the vaccinia virus (Copenhagen strain) and Ectromelia virus, and is encoded in an open reading frame (ORF) known as A39R. The A39R-encoded semaphorin protein is a secreted protein, with no transmembrane domain and no potential membrane linkage. The variola virus ORF, contains sequences that share homology with the vaccinia virus ORF A39R at the nucleotide level and the amino acid level. Another viral semaphorin, AHV-sema, has been found in the Alcelaphine Herpesvirus (AHV).

Mammalian systems are able to develop acquired or specific immunity in response to pathogens. In this manner, mammalian systems are able to specifically recognize and selectively eliminate or protect against foreign microorganisms and molecules (antigens). Controlled manipulation of such a system

would allow one to direct the immune system in particularly efficient or advantageous ways, such as, for example, to specifically target or eliminate diseases that are regulated by the immune system.

One aspect of the immune response involves the interaction between T-lymphocytes, or "T cells," and immune cells known as antigen presenting cells (APCs). T lymphocytes originate from hematopoieti stem cells, and migrate to the thymus gland to mature. Once mature, T cells express a receptor which onl allows recognition of antigens that are processed and complexed with specific cell membrane associated proteins known as Class II major histocompatibility complex (MHC) molecules. When an immunologically naïve T cell encounters a cell presenting a Class II MHC-antigen combination, the T cel proliferates and differentiates into other forms of T cells, including memory T cells, T-helper (T_H) cells, and T cytotoxic (T_C) cells. T_H cells also recognize Class II MHC-antigen combinations. Upon such recognition, the T_H cells are activated to release various cytokines. Under the influence of such T_H cell-derived cytokines, T cells that recognize class I MHC-antigen combinations proliferate and differentiate into cytotoxic T lymphocytes (CTLs). Subsequent steps in the immune cascade follow from these initiating steps.

It is important that regulation of T_H cells be scrupulously specific and controlled, because T_H cell-derived cytokines are required and are in fact critical to both humoral and cell-mediated immune response An important part of T_H cell regulation involves the fact that T_H cells can only recognize antigen in conjunction with class II MHC on the surface of APCs. APC mobility, or chemotaxis, is therefore critical to proper function of this important aspect of the immune system. Control of the ability of an APC to migrate to present antigen to immunologically naive T-cells would allow control of induction of the immune response and, logically, the control of many immune-cell mediated disease pathways. For example, prevention of APC emigration and homing to draining lymph nodes will prevent the induction o the APC-mediated immune responses such as those relative to localized inflammatory responses and autoimmune diseases. Alternatively, promotion of APC migration to draining lymph nodes would accelerate immune responses to specific antigens, thus could be used to promote immunity to, for exampl specific tumor antigens which would promote tumor immunity and prevent tumor cell proliferation.

Another type of immune cell involved in immune system-mediated disease, in particular hypersensitivity disorders such as allergies or urticaria, are mast cells. Mast cells are found throughout connective tissues, particularly near blood and lymphatic vessels. Some tissues, including the skin and mucous membrane surfaces of the respiratory and gastrointestinal tract, contain high concentrations of mast cells per mm^3. When activated, these cells migrate to a site of inflammation and release a variety of pharmacologically active substances, such as histamines and cytokines, from their granules. The release c such substances by mast cells results in the manifestation of a variety of hypersensitivity disorders. Control of mast cell migration would permit the promotion or suppression of release of the pharmacologically active substances they contain, and thus would facilitate control of mast cell-mediated hypersensitivity reactions. For example, promotion of mast cell migration to an area of inflammation in a

patient that suffers from an immune deficiency will allow for promotion of an immune response where needed. Alternatively, suppression of mast cell migration to a site of over-inflammation will prevent deleterious compounding of the immune response. Mast cells are also immune cells involved in the formation of new vasculature. Accordingly, if a method were developed by which mast cell migration could be controlled, it would also allow for the manipulation of angiogenesis.

Recent work has focused on the effect of semaphorins on immune responses. Various investigators have explored the relationship between semaphorins and the immune system and have theorized that semaphorins may somehow function to suppress the immune response in various systems (*see, eg.* PCT Publication No. WO 99/32622, incorporated herein by reference) and, in particular, may operate by inducing cytokine activity (*see*, copending application serial number 08/958,598, incorporated herein by reference, in its entirety). Elucidating other specific relationships between semaphorins and the immune system would prove helpful in the development of useful immuno-therapeutic compositions and methods employing semaphorins. For example, many mammalian diseases, such as auto-immune diseases cancer and hypersensitivity disorders, involve various aspects of the immune response system, and in particular, involve the migration of immune cells. Accordingly, directed or specific control of migration o cells of the immune system would provide a means for therapeutically treating such conditions.

III. SUMMARY OF THE INVENTION

The present invention provides a novel means of using semaphorins or semaphorin receptor polypeptides to treat disorders mediated by the immune system including cancers by controlling immune cell chemotaxis. Preferred semaphorins include AHV Sema, A39R, Sema I, including G-sema I and D-sema-I; Sema II; Sema III; Sema IV; DC Sema; CD100; Z SMF-7; Sema A; Sema B; Sema C; Sema D; Sema E; Sema H; Sema L; Sema W and Sema Y, as well as semaphorin fragments, such as the sema domain of these semaphorins. Preferred semaphorin receptor polypeptides include the Viral-Encoded Semaphorin Receptor ("VESPR") and soluble fragments thereof. Using semaphorins or their receptors, or antibodies to these, the present invention provides compositions and methods for the treatment of immune cell mediated conditions or disorders. Examples of such conditions or disorders include those resulting in an inflammatory response, especially those that are T-cell-mediated, such as auto-immune disorders; cancers such as tumors or diffuse neoplasms, including metastatic neoplasms; hypersensitivity diseases or conditions; conditions associated with deficient vascularization or hypervascularization; and those associated with food allergies.

In one aspect, the present invention provides pharmaceutical compositions and methods for the treatment of a tumor in a mammal. In these embodiments, the invention comprises use of an amount of a semaphorin, or an amount of a soluble semaphorin receptor polypeptide, such as soluble VESPR, or an amount of an antibody to a semaphorin polypeptide receptor such as VESPR, such that administration of an amount of the composition is effective to inhibit progression, or to produce regression or destruction, in whole or in part, of the tumor. Use of such a method or composition according to the present invention ca

also inhibit metastasis of the tumor in the patient. Specific tumors that may be treated with such a composition include virtually any type of tumor, especially those associated with organs, such as liver or colon tumors, or other discrete tissues, such as melanoma. Tumors may be malignant or otherwise cancerous, or benign, so long as they are characterized by an abnormal proliferation of cells.

In another embodiment, the present invention provides a pharmaceutical composition and method for the treatment of a neoplasm in a mammal. When used in this application, the invention comprises an amount of a semaphorin, or an amount of a soluble semaphorin receptor polypeptide such as soluble VESPR or an amount of an antibody to a semaphorin receptor polypeptide such as VESPR, such that administration of an amount of said composition is effective to inhibit progression, or to produce regression or destruction, in whole or in part, of the target neoplasm. Neoplasms that may be treated with the present compositions include metastatic neoplasms, diffuse cancers such as lymphoma or leukemia, or any other manner of systemic proliferation of cancerous cells.

In yet another preferred embodiment, the present invention provides a method or a pharmaceutical composition for the treatment of a mammal with an auto-immune or hypersensitivity disorder or condition, where the auto-immune or hypersensitivity disorder or condition is characterized by an inflammatory response. When used in this application the composition of the present invention includes an amount of a semaphorin, or an amount of a soluble semaphorin receptor polypeptide such as soluble VESPR, or an amount of an antibody to a semaphorin polypeptide receptor such as VESPR, such that administration of an amount of said composition is effective to ameliorate or eliminate, in whole or in part, the inflammation associated with the auto-immune or hypersensitivity disorder or condition. Auto-immune disorders that may be treated with the presently disclosed methods and compositions include rheumatoid arthritis, osteoarthritis, lupus erythematosis, asthma, multiple sclerosis, or any other immune disorder involving immune recognition and targeting of self cells or tissues and resulting in one or more inflammatory responses. Hypersensitivity disorders that may be treated with the presently disclosed compositions and methods include allergies; urticaria, including urticaria in response to pressure, heat, or cold; eczema; and contact dermatitis.

Another aspect of the present invention provides for compositions and methods of promoting or suppressing angiogenesis in a subject. In this aspect, the invention comprises administering to the subject an amount of a semaphorin, or a soluble semaphorin receptor polypeptide such as soluble VESPR or an amount of an antibody to a semaphorin receptor polypeptide such as VESPR, effective to promote or suppress angiogenesis. This method is contemplated to be useful in controlling angiogenesis in a wide variety of circumstances. For example, the disclosed methods and compositions may be used to promote vascular development in patients that have suffered a stroke or severe tissue damage, such as occurs during organ transplantation, heart muscle damage, limb reattachment, organ resection or other trauma. Alternatively, the disclosed compositions and methods, such as by use of a semaphorin or an antibody to

VESPR, can suppress angiogenesis or disrupt vascular development in disfavored tissue such as tumor tissue.

In another aspect, the present invention provides a method and composition, adapted for the treatment of a food allergy or food intolerance in a mammal. In this embodiment, the method or composition employs a semaphorin, or a soluble semaphorin receptor polypeptide such as soluble VESPR, or an antibody to a semaphorin receptor polypeptide such that administration of the composition is effective to ameliorate or eliminate, in whole or in part, the food allergy or intolerance. Exemplary food allergies or intolerances that may be treated with such a composition include allergies to general classes of foods, such as dairy products or wheat products, or to treat allergies or intolerances to specific foods. The present invention also provides a method of treating such food allergies or intolerances, in which the method includes administration of a semaphorin-containing, or soluble semaphorin receptor polypeptide-containing composition to the patient suffering from the food allergy or intolerance. In a preferred embodiment, the composition contains soluble VESPR.

Formulation of any of the presently disclosed compositions may be done by any manner known to those of skill in the art. Such formulations will vary according to variables such as, for example, the needs of the formulator, the intended route of administration, the targeted disease or tissue, and the subject being treated. Specifically, the composition may be formulated in multi-dose containers including additives such as a carrier, other excipients, and a preservative component.

The presently disclosed compositions may also be in virtually any form including an aqueous solution, a suspension, a lyophilized form that may be reconstituted when appropriate, a gel, an aerosol, a transdermal patch or any other form or state convenient for administration to treat the described disorders.

The compositions as described herein may be formulated so that they are contained in a vial, bottle, tube, syringe inhaler or other container for single or multiple administrations. Such containers may be made of glass or a polymer material such as polypropylene, polyethylene, or polyvinylchloride, for example. Preferred containers may include a seal, or other closure system, such as a rubber stopper that may be penetrated by a needle in order to withdraw a single dose and then re-seal upon removal of the needle. All such containers for injectable liquids, lyophilized formulations, reconstituted lyophilized formulations or reconstitutable powders for injection known in the art or for the administration of aerosolized compositions are contemplated for use in the presently disclosed compositions and methods..

In alternative embodiments, the presently disclosed methods and compositions further comprise use or administration of additional active agents such as chemotherapeutic agents, immune suppressants or radiation therapy. For example, agents that may be useful according to the disclosed methods and compositions include virtually any chemotherapeutic agent such as taxotere, doxorubicin, cis-platin, tamoxifen, i-phosphamide, or methotrexate, or variants of any of these compounds. Alternatively or additionally, the presently disclosed methods and compositions may further be used in conjunction with an

immune suppressant, such as a cytokine, IL-4, IL-12, GM-CSF, G-CSF, M-CSF, α-interferon, β-interferon, or γ-interferon.

In an alternative embodiment, the present invention provides a method of screening for a VESPR ligand or other molecule or substance that influences the ability of immune cells to migrate. In other words, a method of identifying VESPR agonists or antagonists. In one embodiment, such a method includes the following steps: (1) contacting a first immune cell comprising a VESPR with a test compound in the presence of a semaphorin; (2) measuring the migration of the first immune cell; and (3) comparing the migration of the first immune cell to migration of a control cell, under comparable conditions in the absence of a test compound. When these steps are followed, the resulting cell migration is observed, with a difference in the motility of the first immune cell relative to the control cell being indicative of an effector of migration of an immune cell. The effector so identified may be either a promoter or a suppressor of immune cell migration.

Alternatively, a VESPR ligand or other molecule or substance that influences immune cell chemotaxis can be identified *via* the following method: (1) contacting an immune cell comprising a VESPR with a test compound in the presence of a known chemokine; (2) measuring the migration of the immune cell; and (3) comparing the migration of the immune cell to migration of a control cell, wherein said control cell is observed under comparable conditions, in the absence of a test compound. When these steps are followed, the resulting cell motility is observed, with a difference in the motility of the immune cell relative to the control cell being indicative of a VESPR ligand or other molecule or substance that functions as an effector of motility of an immune cell. Such screening methods are useful for identifying both promoters and inhibitors of immune cell migration.

In another aspect, the present invention provides a therapeutic package for dispensing to, or for use in dispensing to, a mammal diagnosed with a tumor; a metastatic neoplasm; an auto-immune or hypersensitivity disease or condition characterized at least in part by an inflammatory response; a food allergy or intolerance; or in a mammal in which promotion or inhibition of angiogenesis is desired. Such a therapeutic package will include (1) one or more doses, each such dose comprising at least a semaphorin, a soluble VESPR polypeptide, or an antibody to a semaphorin receptor polypeptide, and (2) a finished pharmaceutical container therefore. In such a package, the amount of semaphorin, soluble VESPR polypeptide, or antibody to a semaphorin receptor polypeptide in the dose or doses is effective, upon administration to the mammal, to inhibit progression, or to produce regression or destruction, in whole or in part, of the tumor or neoplasm; or to ameliorate or eliminate, in whole or in part, the inflammation or food allergy or intolerance; or to promote or inhibit angiogenesis. The finished pharmaceutical container for such a package will contain the dose or doses, and will further contain or comprise labeling directing the use of the package in the treatment of the mammal.

In all aspects of the present invention, it is specifically contemplated that the disclosed compositions and methods are useful to treat the targeted conditions in virtually any mammal, and in particular, they are useful for the treatment of targeted diseases in humans.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: These data show that the semaphorin A39R inhibits both spontaneous migration of monocytes, as well as monocyte migration towards the monocyte-attracting chemokine MCP-1 (as shown in lines -▲- and -◆-). These data also show that VESPR mediates this inhibitory effect of the semaphorin. M460 is a blocking mAb directed against VESPR. According to these results, M460 partially reverses the inhibitory effects of A39R (see line -Δ-). Further confirming the role of VESPR is the data showing that M461, a non-blocking mAb against VESPR and having the same isotype as M460, does not influence the inhibitory effect of the semaphorin (see line -◊-).

Figure 2: These data show the results of the under agarose assays, indicating that migration of immune cells, specifically APCs, in the presence of semaphorin, is inhibited. The bottom frame shows diffuse cells in assay lacking chemokines; the middle frame shows that, in the presence of A39R and MCP1, the cells do not migrate toward the chemo-attractant MCP1, while in the top frame, cells are clearly drawn towards MCP1 in the absence of the semaphorin A39R.

Figure 3: These data show that semaphorins, or semaphorin-receptor complexes influence immune cell motility, but are not lethal to the cells. An increase in fluorescence over time shows that the cells are viable. Cells exposed to A39R remain viable (*see* lines -●-, -□-, -○-, and -◇-).These data confirm that, in cases such as where monocyte migration is inhibited, this immobilization is not the result of cell death.

Figure 4A and Figure 4B: These data show the *in vivo* immune cell migration in the presence of semaphorin, as compared to migration in control animals. Specifically, Langerhans cell migration to regional lymph nodes (upper right quadrant) in A39R-treated animals and control animals is set forth, indicating that, *in vivo*, semaphorins inhibit APC migration to regional lymph nodes (MHC class II positive, FITC positive, compare 9.19% in control animals to 6.21% in semaphorin treated animals).

V. DETAILED DESCRIPTION OF THE INVENTION

Surprisingly, the present invention shows that semaphorins or semaphorin receptors are specifically useful in immune cell regulation because of their influence on immune cell chemotaxis. It is further shown in the present invention that this effect on the motility or migration of immune cells by semaphorins is receptor-mediated.

The following words have the following meanings when used herein:

Antigen Presenting Cell (APC): any cell that can process or present antigenic peptides in association with class II major histocompatibility complex (MHC) molecules. APCs can also deliver a co-stimulatory signal necessary for T-cell activation. APCs may be cells that almost always process or

present class II MHCs, or are involved in T-cell activation such as macrophages; dendritic cells such as, fo example, Langerhans cells and B-cells. Alternatively, some cells may be induced to express class II MHC and to deliver a co-stimulatory signal to T-cells, and thus are APCs if only for a short period of time. These cells include fibroblasts, glial cells, pancreatic beta cells, thymic epithelial cells, thyroid epithelial cells, and vascular endothelial cells. Any cell that either processes or presents class II MHC, or plays a role in T-cell activation, is contemplated to be an APC for purposes of this invention.

Mast Cell: immune system cells involved in various immune system responses, including allergic and hypersensitivity responses, and containing large numbers of cytoplasmic granules comprising pharmacologically active substances such as histamine.

Pharmaceutically acceptable carrier- includes any and all solvents, dispersion media, coatings, antibacterial, isotonic and absorption delaying agents, buffers, excipients, flavorings, binders, lubricants, gels, surfactants and the like, that may be used as a media for a pharmaceutically acceptable substance.

Viral Encoded Semaphorin Protein Receptor (VESPR) Polypeptide: a VESPR polypeptide, as tha term is used herein, is any polypeptide functioning as a receptor for viral semaphorins, for human homologues to viral semaphorins, or for human semaphorins. Specific VESPR-encoding nucleic acid sequences and various forms of VESPR polypeptides are described in copending application S/N 08/958,598, (specifically incorporated herein by reference in its entirety).

Immune cell migration-mediated disease or condition: When used herein this term refers to those diseases or physical states that are dependent upon the migration of immune cells, in particular APCs and mast cells as well as other immune cells the migration of which is influenced by semaphorins or semaphorin receptors. Specific examples of such diseases or conditions include auto-immune diseases or conditions, hypersensitivity diseases or conditions, neoplastic diseases or conditions, food allergies or intolerances, and those diseases or conditions characterized in part by deficient vascularization or hypervascularization.

AutoImmune diseases or conditions: Autoimmune disorders are those involving immune cell recognition and targeting of self cells or tissues and resulting in one or more inflammatory responses. Examples of such diseases or conditions include rheumatoid arthritis, osteoarthritis, lupus erythematosis, asthma, and multiple sclerosis.

Hypersensitivity diseases or conditions: Hypersensitivity disorders are those conditions characterized by either a delayed or an immediate exaggerated response to a foreign stimulus or stimuli. Examples of hypersensitivity diseases or conditions include contact dermatitis; urticaria, including urticari in response to pressure, heat, or cold; eczema; and allergies.

Inflammatory response: The terms inflammatory response and inflammation refer to the pathological process occurring in blood vessels and tissues in response to injury or stimulation caused by a physical, chemical, or biological agent. Inflammation or inflammatory responses are characterized by one

or more of the following non-exhaustive list of symptoms: redness of the affected tissue; heat; swelling; pain; lost function of the tissue or cells affected; contraction of smooth muscle and dilation of capillaries due to release by immune cells of pharmacologically active agents; and development of wheals or hives.

Neoplastic disease or condition: Neoplastic diseases or conditions are those characterized by an abnormal proliferation of cells, generally due to cells continuing to replicate after the stimuli that initiated growth has ceased. Neoplastic diseases or conditions include those in which the abnormal cell proliferation is confined to a specific area or tissue in the body, such as in a confined tumor of an organ or tissue; those that occur in a more diffuse manner throughout the affected subject, such as in the case of leukemia or lymphoma; and those in which the abnormal proliferation of cells spreads or migrates to other tissues or areas of the body, in other words, metastatic cell proliferation.

A. SEMAPHORINS AND SEMAPHORIN RECEPTORS

The present invention is based on the interaction between semaphorins or their agonists or antagonists, and semaphorin receptors, either membrane-bound or soluble.

The present invention contemplates the use of virtually any semaphorin for use in the disclosed compositions and methods. Specific semaphorins that may be used according to the present invention include the viral semaphorins such as Sema IV from both vaccinia and variola viruses, A39R, and AHV-sema. Alternatively those semaphorins cited in the following references are specifically contemplated to be useful according to the present invention: Goodman *et al.*, U.S. Patent No. 5,935,865; Dodd and Schuchardt, 1995); EP 945,505; EP 933,425; EP 960,888; EP 960,937; and WO 99/32622. Such semaphorins include, but are not limited to: Sema I, including G-sema I and D-sema-I; Sema II; Sema III; Sema IV; DC Sema; CD100; Z SMF-7; Sema A; Sema B; Sema C; Sema D; Sema E; Sema H; Sema L; Sema W and Sema Y.

Additionally, it is specifically contemplated that fragments of these semaphorins, such as, for example, the fragments including the sema domain or the active domain will be useful according to the present invention, as will fusion proteins encompassing a semaphorin or semaphorin fragment. For example, the DC sema fusion proteins described in co-pending PCT patent application No. PCT/US99/09831, which was filed as a provisional in the United States, as Provisional Application Serial No. 60/085,497 (specifically incorporated herein by reference) will be useful. For additional semaphorins that can be used in the presently disclosed compositions and methods, *see* Bamberg, *et.al.* Cell, 97:551 and United States Patent No. 5,935,865 to Goodman *et al.* Nucleic acid sequences encoding the semaphorins or semaphorin fragments of the present invention, are also specifically contemplated to be useful in the disclosed compositions and methods.

Semaphorin receptors or semaphorin receptor polypeptides are known to be various molecules including those identified as neuropilins or plexins (*see, Comeau, et al.*, April 1998). Plexins contain a "sema" domain that is related to the sema domain of semaphorins themselves, part of which constitutes a series of two or three cystein repeat sequences in the extracellular domain of plexins. Plexins are distinct

from semaphorins, however, in a variety of respects. For example, in their intracellular domain, plexins are strongly homologous throughout the family of plexins, and contain well-conserved amino acid motifs that are not found in semaphorins.

Semaphorin receptors of the present invention are those plexin polypeptide sequences that can interact with a semaphorin or a semaphorin fragment, to influence immune cell migration. Exemplary semaphorin receptor polypeptides include full-length plexin receptor polypeptides as well as homologues or fragments, such as the soluble extra cellular domain or the sema domain of such plexin receptor polypeptides. An exemplary Plexin receptor is the Viral Encoded Semaphorin Protein Receptor "VESPR," (described in copending patent application serial number 08/958,598). Specifically the amino acid sequence of SEQ ID NO:2 is useful as a semaphorin receptor polypeptide in the presently disclosed compositions and methods, as are the homologues and variants of polypeptides of SEQ ID NO:2. In particular, the present invention contemplates the use of fragments of VESPR, such as the soluble fragmer of VESPR. An example of a soluble VESPR receptor polypeptide includes amino acids 1-944 of SEQ ID NO:2. In addition, truncated soluble VESPR proteins comprising less that the entire extracellular domain are included in the invention, *e.g.*, amino acids 35-944.

Also encompassed within the present invention are the nucleic acid sequences encoding such useful VESPR receptor polypeptides and polypeptide fragments. Nucleic acid sequences encoding the semaphorin receptors or receptor fragments are also within the scope of the presently disclosed compositions and methods. Particularly preferred nucleic acid sequences include the polynucleotide sequence of SEQ ID NO:1; and those segments of SEQ ID NO:1 that encode the soluble fragments of VESPR outlined above.

The semaphorin or semaphorin receptor polypeptides of the invention may be membrane bound o they may be secreted and thus soluble. Soluble polypeptides are capable of being secreted from the cells i which they are expressed. In general, soluble polypeptides may be identified (and distinguished from non soluble membrane-bound counterparts) by separating intact cells which express the desired polypeptide from the culture medium, *e.g.*, by centrifugation, and assaying the medium (supernatant) for the presence of the desired polypeptide. The presence of polypeptide in the medium indicates that the polypeptide was secreted from the cells and thus is a soluble form of the protein.

In one embodiment, the soluble polypeptides and fragments thereof comprise all or part of the extracellular domain, but lack the transmembrane region that would cause retention of the polypeptide on cell membrane. A soluble polypeptide may include the cytoplasmic domain, or a portion thereof, as long as the polypeptide is secreted from the cell in which it is produced. In general, the use of soluble forms is advantageous for certain applications. Purification of the polypeptides from recombinant host cells is facilitated, since the soluble polypeptides are secreted from the cells. Further, soluble polypeptides are generally more suitable for intravenous administration.

The invention also provides polypeptides and fragments of the extracellular domain that retain a desired biological activity. Particular embodiments are directed to polypeptide fragments that retain the ability to interact with the semaphorin receptor or ligand to influence immune cell migration. Such a fragment may be a soluble polypeptide, as described above. In another embodiment, the polypeptides and fragments advantageously include regions that are conserved in the semaphorin family, in the case of semaphorins; or regions that are conserved in the plexin family in the case of the semaphorin receptors; or include the sema domain of either.

Also provided herein are polypeptide fragments comprising at least 20, or at least 30, contiguous amino acids of the sequence of SEQ ID NO:2. Fragments derived from the cytoplasmic domain find use in studies of signal transduction, and in regulating cellular processes associated with transduction of biological signals. Polypeptide fragments also may be employed as immunogens, in generating antibodies.

Naturally occurring variants as well as derived variants of the polypeptides and fragments are provided herein. Variants may exhibit amino acid sequences that are at least 80% identical. Also contemplated are embodiments in which a polypeptide or fragment comprises an amino acid sequence that is at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, or at least 99.9% identical to the preferred polypeptide or fragment thereof. Percent identity may be determined by visual inspection and mathematical calculation. Alternatively, the percent identity of two protein sequences can be determined by comparing sequence information using the GAP computer program, based on the algorithm of Needleman and Wunsch (J. Mol. Bio. 48:443, 1970) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The preferred default parameters for the GAP program include: (1) a scoring matrix, blosum62, as described by Henikoff and Henikoff (Proc. Natl. Acad. Sci. USA 89:10915, 1992); (2) a gap weight of 12; (3) a gap length weight of 4; and (4) no penalty for end gaps. Other programs used by one skilled in the art of sequence comparison may also be used.

The variants of the invention include, for example, those that result from alternate mRNA splicing events or from proteolytic cleavage. Alternate splicing of mRNA may, for example, yield a truncated but biologically active protein, such as a naturally occurring soluble form of the protein. Variations attributable to proteolysis include, for example, differences in the N- or C-termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids from the protein (generally from 1-5 terminal amino acids). Proteins in which differences in amino acid sequence are attributable to genetic polymorphism (allelic variation among individuals producing the protein) are also contemplated herein.

Additional variants within the scope of the invention include polypeptides that may be modified to create derivatives thereof by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives may be prepared by linking the chemical moieties to functional groups on amino acid side chains or at the N-

terminus or C-terminus of a polypeptide. Conjugates comprising diagnostic (detectable) or therapeutic agents attached thereto are contemplated herein, as discussed in more detail below.

Other derivatives include covalent or aggregative conjugates of the polypeptides with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. Examples of fusion proteins are discussed below in connection with oligomers. Further, fusion proteins can comprise peptides added to facilitate purification and identification. Such peptides include, for example, poly-His or the antigenic identification peptides described in U.S. Patent No. 5,011,912 and in Hopp et al., *Bio/Technology* 6:1204, 1988. One such peptide is the FLAG® peptide, Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys, which is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. A murine hybridoma designated 4E11 produces a monoclonal antibody that binds the FLAG® peptide in the presence of certain divalent metal cations, as described in U.S. Patent 5,011,912, hereby incorporated by reference. The 4E11 hybridoma cell line has been deposited with the American Type Culture Collection under accession no. HB 9259. Monoclonal antibodies that bind the FLAG® peptide are available from Eastman Kodak Co., Scientific Imaging Systems Division, New Haven, Connecticut.

Among the variant polypeptides provided herein are variants of native polypeptides that retain the native biological activity or the substantial equivalent thereof. One example is a variant that binds with essentially the same binding affinity as does the native form. Binding affinity can be measured by conventional procedures, *e.g.*, as described in U.S. Patent No. 5,512,457 and as set forth below.

Variants include polypeptides that are substantially homologous to the native form, but which have an amino acid sequence different from that of the native form because of one or more deletions, insertions or substitutions. Particular embodiments include, but are not limited to, polypeptides that comprise from one to ten deletions, insertions or substitutions of amino acid residues, when compared to a native sequence.

A given amino acid may be replaced, for example, by a residue having similar physiochemical characteristics. Examples of such conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another; substitutions of one polar residue for another, such as between Lys and Arg, Glu and Asp, or Gln and Asn; or substitutions of one aromatic residue for another, such as Phe, Trp, or Tyr for one another. Other conservative substitutions, *e.g.*, involving substitutions of entire regions having similar hydrophobicity characteristics, are well known.

Similarly, the DNAs of the invention include variants that differ from a native DNA sequence because of one or more deletions, insertions or substitutions, but that encode a biologically active polypeptide.

The invention further includes polypeptides of the invention with or without associated native-pattern glycosylation. Polypeptides expressed in yeast or mammalian expression systems (*e.g.*, COS-1 or COS-7 cells) can be similar to or significantly different from a native polypeptide in molecular weight and

glycosylation pattern, depending upon the choice of expression system. Expression of polypeptides of the invention in bacterial expression systems, such as *E. coli*, provides non-glycosylated molecules. Further, a given preparation may include multiple differentially glycosylated species of the protein. Glycosyl groups can be removed through conventional methods, in particular those utilizing glycopeptidase. In general, glycosylated polypeptides of the invention can be incubated with a molar excess of glycopeptidase (Boehringer Mannheim).

Correspondingly, similar DNA constructs that encode various additions or substitutions of amino acid residues or sequences, or deletions of terminal or internal residues or sequences are encompassed by the invention. For example, N-glycosylation sites in the polypeptide extracellular domain can be modified to preclude glycosylation, allowing expression of a reduced carbohydrate analog in mammalian and yeast expression systems. N-glycosylation sites in eukaryotic polypeptides are characterized by an amino acid triplet Asn-X-Y, wherein X is any amino acid except Pro and Y is Ser or Thr. Appropriate substitutions, additions, or deletions to the nucleotide sequence encoding these triplets will result in prevention of attachment of carbohydrate residues at the Asn side chain. Alteration of a single nucleotide, chosen so that Asn is replaced by a different amino acid, for example, is sufficient to inactivate an N-glycosylation site. Alternatively, the Ser or Thr can by replaced with another amino acid, such as Ala. Known procedures for inactivating N-glycosylation sites in proteins include those described in U.S. Patent 5,071,972 and EP 276,846, hereby incorporated by reference.

In another example of variants, sequences encoding Cys residues that are not essential for biological activity can be altered to cause the Cys residues to be deleted or replaced with other amino acids, preventing formation of incorrect intramolecular disulfide bridges upon folding or renaturation.

Other variants are prepared by modification of adjacent dibasic amino acid residues, to enhance expression in yeast systems in which KEX2 protease activity is present. EP 212,914 discloses the use of site-specific mutagenesis to inactivate KEX2 protease processing sites in a protein. KEX2 protease processing sites are inactivated by deleting, adding or substituting residues to alter Arg-Arg, Arg-Lys, and Lys-Arg pairs to eliminate the occurrence of these adjacent basic residues. Lys-Lys pairings are considerably less susceptible to KEX2 cleavage, and conversion of Arg-Lys or Lys-Arg to Lys-Lys represents a conservative and preferred approach to inactivating KEX2 sites.

Encompassed by the invention are oligomers or fusion proteins that contain semaphorin or semaphorin receptor polypeptides. Such oligomers may be in the form of covalently-linked or non-covalently-linked multimers, including dimers, trimers, or higher oligomers. As noted above, preferred polypeptides are soluble and thus these oligomers may comprise soluble polypeptides. In one aspect of the invention, the oligomers maintain the binding ability of the polypeptide components and provide therefor, bivalent, trivalent, etc., binding sites.

One embodiment of the invention is directed to oligomers comprising multiple polypeptides joined *via* covalent or non-covalent interactions between peptide moieties fused to the polypeptides. Such

peptides may be peptide linkers (spacers), or peptides that have the property of promoting oligomerization. Leucine zippers and certain polypeptides derived from antibodies are among the peptides that can promote oligomerization of the polypeptides attached thereto, as described in more detail below.

As one alternative, an oligomer is prepared using polypeptides derived from immunoglobulins. Preparation of fusion proteins comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, *e.g.*, by Ashkenazi et al. (*PNAS USA* 88:10535, 1991); Byrn et al. (*Nature* 344:677, 1990); and Hollenbaugh and Aruffo ("Construction of Immunoglobulin Fusion Proteins", in *Current Protocols in Immunology*, Suppl. 4, pages 10.19.1 - 10.19.11, 1992).

One embodiment of the present invention is directed to a dimer comprising two fusion proteins created by fusing a polypeptide of the invention to an Fc polypeptide derived from an antibody. A gene fusion encoding the polypeptide/Fc fusion protein is inserted into an appropriate expression vector. Polypeptide/Fc fusion proteins are expressed in host cells transformed with the recombinant expression vector, and allowed to assemble much like antibody molecules, whereupon interchain disulfide bonds form between the Fc moieties to yield divalent molecules.

The term "Fc polypeptide" as used herein includes native and mutein forms of polypeptides made up of the Fc region of an antibody comprising any or all of the CH domains of the Fc region. Truncated forms of such polypeptides containing the hinge region that promotes dimerization are also included. Preferred polypeptides comprise an Fc polypeptide derived from a human IgG1 antibody.

One suitable Fc polypeptide, described in PCT application WO 93/10151 (hereby incorporated by reference), is a single chain polypeptide extending from the N-terminal hinge region to the native C-terminus of the Fc region of a human IgG1 antibody. Another useful Fc polypeptide is the Fc mutein described in U.S. Patent 5,457,035 and in Baum et al., (*EMBO J.* 13:3992-4001, 1994) incorporated herein by reference. The amino acid sequence of this mutein is identical to that of the native Fc sequence presented in WO 93/10151, except that amino acid 19 has been changed from Leu to Ala, amino acid 20 has been changed from Leu to Glu, and amino acid 22 has been changed from Gly to Ala. The mutein exhibits reduced affinity for Fc receptors.

The above-described fusion proteins comprising Fc moieties (and oligomers formed therefrom) offer the advantage of facile purification by affinity chromatography over Protein A or Protein G columns.

In other embodiments, the polypeptides of the invention may be substituted for the variable portion of an antibody heavy or light chain. If fusion proteins are made with both heavy and light chains of an antibody, it is possible to form an oligomer with as many as four semaphorin or semaphorin receptor extracellular regions. Alternatively, the oligomer is a fusion protein comprising multiple polypeptides, with or without peptide linkers (spacer peptides). Among the suitable peptide linkers are those described in U.S. Patents 4,751,180 and 4,935,233, which are hereby incorporated by reference. A DNA sequence encoding a desired peptide linker may be inserted between, and in the same reading frame as, the DNA

sequences of the invention, using any suitable conventional technique. For example, a chemically synthesized oligonucleotide encoding the linker may be ligated between the sequences. In particular embodiments, a fusion protein comprises from two to four soluble semaphorin or semaphorin receptor polypeptides, separated by peptide linkers.

Another method for preparing the oligomers of the invention involves use of a leucine zipper. Leucine zipper domains are peptides that promote oligomerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, 1988), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Zipper domains (also referred to herein as an oligomerizing, or oligomer-forming, domain) and their use are well-known in the art.

Alternatively, agonists or antagonists of semaphorins, including antibodies (both monoclonal and polyclonal) viruses and small molecule agonists or antagonists will be useful according to the present invention. Further contemplated to be useful in the presently disclosed invention are agonists or antagonists of the interaction between a semaphorin receptor polypeptide such as VESPR and a semaphorin. For example, antibodies to semaphorin receptor polypeptides, such as VESPR antibodies, are specifically contemplated to be useful according to the present invention. Methods of identifying and utilizing such agonists or antagonists are well-known in the art.

B. SEMAPHORINS INFLUENCE MIGRATION OF IMMUNE CELLS

The present invention is concerned with the receptor-mediated influence of semaphorins, semaphorin agonists such as agonistic antibodies, or semaphorin antagonists, on cells of the immune system such as antigen presenting cells and mast cells. Two techniques prove particularly helpful in elucidating the mechanism of this influence: the transwell migration assay (Boyden Chamber, Boyden S.J.; Baum, J. *et. al.*, (1971); Keller, H.U. *et. al.*; Snyderman, R. *et. al.*; Zigmond, S.H. *et. al.*; Gallin, J.I., *et. al*, and the under-agarose assay (Nelson, R.D. *et. al.*. Using these assays, the present invention shows that semaphorins exhibit profound effects on cell migration, specifically on the migration of APCs and mast cells, either when used alone in culture, or in combination with known chemoattractants.

A39R, a viral semaphorin, proves a useful semaphorin to show this effect. In the transwell migration assay, human or mouse APCs are loaded with a fluorescent dye and seeded into the upper chamber of a transwell apparatus. This apparatus consists of two chambers stacked vertically, and separated by a porous filter. Medium, chemoattractants (such as chemokines), or other chemokinetic factors are placed in the bottom chamber, and the migration of APCs across the porous membrane is measured on a fluorescent plate reader in real time. In this assay, the presence of A39R alone in the lower chamber abolishes all spontaneous migration of antigen presenting cells through the membrane, and when placed in the bottom chamber along with chemokines that facilitate the movement of APCs, A39R

significantly interferes with the movement of APC across the membrane in response to the chemoattractant.

C. SEMAPHORINS ALLOW MANIPULATION OF IMMUNE CELL MIGRATION *IN VITRO* AND *IN VIVO*

The present invention further provides a means of manipulating APC migration when a semaphorin is administered along a "gradient." To illustrate this, the effects of A39R on APC migration, when the semaphorin is presented to cells along a "gradient" (concentration) were determined, via the under-agarose assay. In this experiment, a petri dish is coated with medium-containing agarose. When this layer hardens, a line of three small, uniform holes are punched in the agarose. Cells are seeded into the center well, chemoattractants or other chemokinetic reagents are seeded into an adjacent well, and medium is seeded into the third well as a control. The assay is monitored over a period of time by light microscopy. Responsive APC move in a directional fashion, under the agarose, toward a chemoattractant and can be photographed using photomicroscopy over a period of time. In these assays, A39R dramatically interfered with the migration of the APC toward a chemoattractant, when placed in the same well, at the same time, as the chemoattractant. Additionally, A39R placed alone in a well adjacent to seeded APC caused significant APC movement, but in either a non-directional fashion, or in a direction that was away from the source of A39R. Results are shown in Figure 2.

In both the transwell assay as well as the under-agarose assay, appropriate controls consisting of heat inactivated, or heterologous proteins were included. In the case of human monocytes, the experiments have been performed on no less than five donors, in excess of three times for each donor. Experiments using blocking monoclonal antibodies against the Immunex proprietary molecule VESPR, the receptor for the semaphorin A39R, have indicated that the effects of the viral semaphorin on cell migration are mediated through VESPR.

The present invention specifically contemplates that the disclosed compositions and methods will be effective when administered to a patient. *In vivo* cell migration assays were performed to illustrate this utility. Drawing on similar experiments to assess the role of cytokines such as IL-10 or TNF in Langerhans cell migration, *in vivo* assays were constructed. Specifically, Langerhans cells provided a useful experimental system to confirm that semaphorins elicit a local effect on antigen presenting cell migration. Results showed that local administration of semaphorin effects skin APC migration to regional lymph nodes. The results of such assays are shown in Figure 4A and Figure 4B.

Given this result, the present invention shows that the binding or other interaction between a semaphorin, such as the viral semaphorin A39R, its human homologue, or any agonistic antibody or small molecule, and VESPR, inhibits the movement of an APC towards any area where it might encounter a Tcell, which would then recognize the presented antigen, or encounter an other responsive cell that might be effected by monokine production. The T cell or other responsive cell would be prevented from responding according to its immune system role, such as by proliferating, producing cytokines, promoting

the function of B-cells, other T cells, or Natural Killer (NK) cells, macrophages or any other cell that responds to cytokines. The present invention further teaches that mast cell migration is influenced by the interaction between a semaphorin such as the viral semaphorin A39R, its human homologue, or any agonistic antibody or small molecule, and VESPR. Thus, mast cell migration to site of inflammation, and release of the granular contents of the mast cell can be controlled *via* manipulation of the semaphorin-receptor interaction.

Specific examples of how administration of a semaphorin or semaphorin receptor or antagonist or agonist of either might be used as an immunotherapeutic includes treatment of an auto-immune or hypersensitivity diseases or conditions; treatment of neoplastic diseases or conditions; treatment of food allergies or intolerances and promotion or suppression of angiogenesis.

1. Semaphorins and Semaphorin Receptors Can Be Used To Treat Auto-immune or Hypersensitivity Disorders

Using a semaphorin to inhibit APC migration, would allow minimization of the amount of self antigen presented to T cells, thus inhibiting proliferation of self-reactive T cells, and inhibiting production of pro-inflammatory cytokines. Any semaphorin agonist identified by the processes disclosed herein, would function equally well in this manner. Additionally or alternatively, the interaction between semaphorins and their receptors, or agonists or antagonists of this interaction can be used to influence mast cell migration to treat autoimmune or hypersensitivity disorders or conditions such as various forms of arthritis, lupus, multiple sclerosis, allergies, urticaria, eczema or dermatitis. For example, an agonistic antibody to VESPR, or a soluble semaphorin that binds VESPR can be administered to interfere with the migration of mast cells to sites of inflammation, thus reducing the allergic, urticaric, asthmatic or other hypersensitivity or autoimmune response.

Alternatively, as another example, targeted delivery of a soluble version of VESPR can also facilitate the migration of mast cells into an area of inflammation to promote a chemokine driven immune response. This sort of treatment would be useful in instances where the immune response is deficient or suppressed, such as when the subject suffers from an immunodeficiency disease or has suppressed immune response due to concurrent or previous therapeutic regimens, such as in the case of patients undergoing cancer chemotherapy.

2. Semaphorins and Semaphorin Receptors Can Be Used To Treat Neoplastic Diseases

Additionally, semaphorins or semaphorin receptors, or agonists or antagonists of their interaction may be used to regulate the immune cascade and treat cancer or other neoplastic diseases. One form of such immunotherapy would involve, for example, presentation of the naturally-occurring ligand for the semaphorin receptor, rather than the viral semaphorin. The naturally occurring ligand would inhibit migration of tumor cells bearing the VESPR thus inhibiting the movement or metastasis of the transformed tumor cells expressing the VESPR. Such a method may be preferred where it is desired to temporarily arrest the endogenous tumor response and to minimize chances of metastasis, while alternative and

additional cancer therapies, such a chemotherapeutic agents or radiation therapy, are directed to the localized tumor. A similar process allows for treatment of diffuse cancers, such as lymphoma and leukemia.

Alternatively, a useful cancer immunotherapy would include administering a VESPR antagonist or competitive inhibitor, for example, a soluble VESPR, in those cancers in which the cancerous cells overexpress semaphorin ligands that inhibit APC migration. The naturally occurring ligand would inhibit migration of APCs bearing tumor antigen, thus the administration of an antagonist would allow for increased presentation of tumor antigens to T cells, resulting in increased tumor cell immunity as well as an increased targeting of tumor cells by the immune system. Such a cancer immunotherapy would function by competitively inhibiting semaphorin interaction with VESPR, thereby reducing the inhibitory effect of semaphorins on APC migration.

Another indication for antagonizing VESPR might be at the level of antibody production. Antibodies are made after APCs activate T_H cells, which then provide cytokines that expand B cell populations, causing them to differentiate and to make mature antibodies. As the naturally occurring ligand for VESPR inhibits the migration of APC, the humoral (or antibody response in the presence of the ligand) may be lower than it could be. Thus, antagonizing that inhibition (by, for example, administering a soluble VESPR) might allow a more robust antibody response to a given antigen, or encourage the presentation of rare immunodominant or protective epitopes which would be useful in treating infections or would be useful in an adjuvant-like capacity for bolstering immune response to vaccines.

3. Semaphorins and Semaphorin Receptors Can Be Used To Treat Food Intolerances

Another aspect of the invention includes promotion of presentation of food antigens. Normal intake of food exposes the immune system to a wide array of foreign antigens. As the naturally occurring ligand for VESPR diminishes APC cell migration, antagonizing that interaction may lead to increased presentation of food antigens thereby promoting tolerance to the various food antigens. Such antagonism would be indicated in the treatment of individuals exhibiting food allergies or other food intolerances.

4. Semaphorins and Semaphorin Receptors Can Be Used To Promote or Suppress Angiogenesis

Mast cells are involved in the formation of new blood vessel formation from endothelium, or angiogenesis. Thus, semaphorins, semaphorin receptors, or agonists or antagonists of the interaction between the two can be used to manipulate angiogenesis in a subject. Such methods or compositions can be used to treat a subject with an immune cell migration-mediated disease or condition characterized in part by deficient vascularization or hypervascularization. Examples of diseases or conditions characterized by deficient vascularization include ischemic tissue or other traumatized tissue, stroke, organ transplantation and the like. Examples of diseases or conditions characterized by hypervascularization include various tumors. Treatment of such conditions can be achieved by, for example, delivering agonists of VESPR, such as an antibody to VESPR or a semaphorin, particularly in a targeted fashion, to help

disrupt the development of new blood vessels needed to feed the tumor. Inhibition of vasculature necessary for tumor growth will assist in inhibition of progression of the tumor, or even regression or destruction of the tumor. Alternatively, delivering a soluble version of VESPR will facilitate the migration of mast cells to areas requiring new vessel formation, such as, for example in patients that have suffered a stroke or have sustained severe tissue damage from organ transplantation, limb reattachment, organ resection or other trauma, ischemia, or the like.

D. IDENTIFICATION OF EFFECTORS OF IMMUNE CELL MIGRATION

The interaction between semaphorins and semaphorin receptors can also be used, according to the present invention, to identify additional agents that influence immune cell migration. Such agents may be VESPR ligands, or may be agonists or antagonists of the interaction between VESPR and semaphorins. Examples of such screening assays include a method of screening for a VESPR ligand or other molecule or substance that influences the ability of immune cells to migrate by executing the following steps: (1) contacting a first immune cell comprising a VESPR with a test compound in the presence of a semaphorin; (2) measuring the migration of the first immune cell; and (3) comparing the migration of the first immune cell to migration of a control cell, under comparable conditions in the absence of a test compound. When these steps are followed, the resulting cell migration is observed, with a difference in the motility of the first immune cell relative to the control cell being indicative of an effector of migration of an immune cell. The effector so identified may be either a promoter or a suppressor of immune cell migration.

Alternatively, a VESPR ligand or other molecule or substance that influences immune cell chemotaxis can be identified *via* the following method: (1) contacting an immune cell comprising a VESPR with a test compound in the presence of a known chemokine; (2) measuring the migration of the immune cell; and (3) comparing the migration of the immune cell to migration of a control cell, wherein said control cell is observed under comparable conditions, in the absence of a test compound. When these steps are followed, the resulting cell motility is observed, with a difference in the motility of the immune cell relative to the control cell being indicative of a VESPR ligand or other molecule or substance that functions as an effector of motility of an immune cell. Such screening methods are useful for identifying both promoters and inhibitors of immune cell migration.

Compounds that can be screened in accordance with the invention include but are not limited to peptides (*e.g.*, polypeptides such as proteins, and small peptides), non-peptide organic molecules, and inorganic molecules. A number of compound libraries are commercially available from companies such as Pharmacopeia, Argule, Enzymed, Sigma, Aldrich, Maybridge, Trega and PanLabs, to name just a few sources. One can also screen libraries of known compounds, including natural products or synthetic chemicals, and biologically active materials for compounds that are effectors of immune cell migration.

E. ROUTES OF SEMAPHORIN ADMINISTRATION

The presently disclosed compositions and methods may utilize both oral and non-oral administration routes including, for example, by injection *via* the intradermal, subcutaneous, and intravenous routes; by transdermal delivery; by inhalation or buccal delivery, or by ingestion of tablets or capsules. In certain aspects of the invention, compounds may be administered locally to tumors or tumor cells. Local delivery to a solid tumor can be by intratumoral injection, injection circumferential to the tumor, injection into the vascular supply to the tumor, injection into the vasculature or lymphatics to effect infusion of the region containing the tumor, regional perfusion by use of an extracorporeal circuit, or other delivery methods that effect a localized concentration of compound in the tumor or region containing the tumor. Local and regional delivery of compounds to metastasized tumor cells and/or post-resection macroscopic or microscopic residual tumors or tumor cells can be by local injection into the tissue, injection into the vasculature or lymphatics to effect regional infusion, regional perfusion by use of an extracorporeal circuit, or other delivery methods that effect a localized concentration of compound in a region or tissue.

F. DOSAGES

The optimal daily dose of semaphorin, VESPR or soluble VESPR, or of an agonist or antagonist of one of these, alone or in combination, useful for the purposes of the present invention is determined by methods known in the art. For example, dosages can be determined based on the severity of the disease or condition being treated, the condition of the subject to whom treatment is being given, the desired degree of therapeutic response, and any concomitant therapies being administered to the subject. Ordinarily, however, administration will be such that a serum level of between about 100ng/ml to about 100μg/ml of semaphorin, semaphorin receptor, or agonist or antagonist of either, is achieved. Preferred doses will achieve blood serum levels of between 500ng/ml and 1μg/ml. The dose can be administered in a single or multiple dosage regimen, or may be by a method that allows for a continuous release of relatively small amounts of the active ingredient from a single dosage unit, such as by a transdermal patch or ingested extended release capsule, over the course of one or more days.

To determine when inhibition or retardation of the various target diseases or conditions, or when amelioration, regression or destruction of the targeted diseases or conditions has been achieved, any of the following can be considered: improvement in patient condition or quality of life; increased longevity of life; decreased pain; decreased severity of symptoms of the targeted disease or condition; retardation of abnormal tissue growth or metastases; increase in desired tissue growth, such as in the case of promotion of angiogenesis; increased airway or lung capacity in the case of asthma or other targeted respiratory diseases; increased circulatory capacity in the case of vascular applications of the invention; decreased food intolerance; and the like. Any of these endpoints as well as others may be considered to determine the

effectiveness of the therapy, and may be measured or determined by patient self-evaluation; objective screening; or by diagnostic testing such as by X-ray, CT or PET scanning or the like.

G. SEMAPHORIN-CONTAINING FORMULATION COMPONENTS

In addition to a semaphorin, optimal disclosed compositions and methods will vary according to factors such as, for example, the target tissues, target disease, amount of time the formulation will be stored, conditions under which it will be stored and used, including the dosage form of the composition, and the particular patient population to which it may be administered. Adjustments to the formulation by adjusting constituents of the formulations and their relative concentrations, may be made as needed according to the needs of the formulator, administrator or patient. Additional constituent elements of the multi-dose formulations of the present invention may include water, a buffer, a pH-adjusting agent, a surfactant or anti-adsorbant, a wetting agent, a gelling agent, a drying agent, an osmolality adjusting agent, or virtually any other additive or carrier, depending upon the desired dosage form and intended route of administration.

Formulation characteristics that may be modified include, for example, the pH and the osmolality. For example, it may be desired to achieve a formulation that has a pH and osmolality similar to that of human blood or tissues to facilitate the formulation's effectiveness when administered parenterally. Alternatively, to promote the effectiveness of the disclosed compositions when administered via other administration routes, alternative characteristics may be modified.

Buffers are useful in the present invention for, among other purposes, manipulation of the total pH of the pharmaceutical formulation (especially desired for parenteral administration). A variety of buffers known in the art may be used in the present formulations, such as various salts of organic or inorganic acids, bases, or amino acids, and including various forms of citrate, phosphate, tartrate, succinate, adipate, maleate, lactate, acetate, bicarbonate, or carbonate ions. Particularly advantageous buffers for use in parenterally administered forms of the presently disclosed compositions in the present invention include sodium or potassium buffers, particularly sodium phosphate. Depending upon the dosage form and intended route of administration it may alternatively be advantageous to use buffers in different concentrations or to use other additives to adjust the pH of the composition. Useful pH ranges for compositions of the present invention include a pH of about 2.0 to a pH of about 12.0, again, depending upon various factors such as the intended route of administration.

It may also be advantageous to employ surfactants in the presently disclosed formulations. Surfactants or anti-adsorbants that prove useful include polyoxyethylenesorbitans, polyoxyethylenesorbitan monolaurate, polysorbate-20, such as Tween-20™, polysorbate-80, hydroxycellulose, and genapol. By way of example, when any surfactant is employed in the present invention to produce a parenterally administrable composition, it is advantageous to use it in a concentration of about 0.01 to about 0.5 mg/ml.

Additionally, such compositions can be complexed with polyethylene glycol (PEG), metal ions, or incorporated into polymeric compounds such as polyacetic acid, polyglycolic acid, hydrogels, dextran, *etc.*,

or incorporated into liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts or spheroblasts. Such compositions will influence the physical state, solubility, stability, rate of *in vivo* release and rate of *in vivo* clearance, and are thus chosen according to the intended application.

Additional useful additives are readily determined by those of skill in the art, according to particular needs or intended uses of the compositions and formulator. One such particularly useful additional substance is sodium chloride, which is useful for adjusting the osmolality of the formulations to achieve the desired resulting osmolality. Particularly preferred osmolalities for parenteral administration of the disclosed compositions are in the range of about 270 to about 330 mOsm/kg.

H. PREPARATION OF THE COMPOSITIONS

The formulations described herein may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose or polyoxyethylenesorbitans. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride as described above. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate or gelatin. Other agents that may be employed include, but are not limited to lecithin, urea, ethylene oxide, propylene oxide, hydroxypropylcellulose, methylcelluylose, or polyethylene glycol.

Aqueous compositions (inocula) as described herein may include an effective amount of a desired pharmacologically active agent dissolved or dispersed in a pharmaceutically acceptable aqueous medium. Such compositions are also referred to as inocula. The use of pharmaceutically acceptable carrier media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions as described above.

A semaphorin used in the present invention may be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and those that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

The therapeutic compositions of the present invention are advantageously administered in the form of injectable compositions either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. Alternatively, the compositions of the present invention may be administered as inhalants in an aerosolized form. Depending upon the needs of the

formulator, administrator, or the subject of the treatment, the presently disclosed compositions may take virtually any form including liquid, suspension, emulsion, solution, oil, mixture, cream, ointment, gel, suppository, semi-solid, aerosol, powder, tablet, or capsule. A typical composition comprises a pharmaceutically acceptable carrier. For instance, the composition may contain 10 mg, 25 mg, 50 mg or up to about 100 mg of human serum albumin per milliliter of phosphate buffered saline.

The formulations as described herein may be contained in a vial, bottle, tube, syringe inhaler or other container for single or multiple administrations. Such containers may be made of glass or a polymer material such as polypropylene, polyethylene, or polyvinylchloride, for example. Preferred containers may include a seal, or other closure system, such as a rubber stopper that may be penetrated by a needle in order to withdraw a single dose and then re-seal upon removal of the needle. All such containers for injectable liquids, lyophilized formulations, reconstituted lyophilized formulations or reconstitutable powders for injection known in the art or for the administration of aerosolized compositions are contemplated for use in the presently disclosed compositions and methods.

VI. EXAMPLES

The following examples are illustrative only and are not intended to limit the scope of the invention.

A. EXAMPLE 1: SEMAPHORINS INFLUENCE IMMUNE CELL CHEMOTAXIS AS SHOWN BY ASSAY USING FLUOROBLOK INSERTS

To assay the effect, *in vitro*, of semaphorins in immune cell migration, human monocytes from apparently healthy donors were separated by counter current centrifugation (elutriation) from freshly drawn leukophoresis packs using a Beckman J-6M/E centrifuge (Beckman Instruments Inc.; Palo Alto, CA) and aliquots stored under Liquid Nitrogen. Monocyte samples were thawed rapidly at 37°C and added to 30 ml of ice cold RPMI 1640, 5% FBS (Gibco BRL; Rockville, MD) in a FALCON 50-ml conical tube (Becton Dickinson Labware; Franklin Lakes, NJ) then centrifuged at 1400 rpm, 5 minutes, at room temperature.

Monocytes were resuspended in 30 ml, 37°C Hanks Balanced Salt Solution without phenol red (HBSS w/out red) (Gibco BRL; Rockville, MD), and counted by Trypan Blue (Gibco BRL, Rockville, MD) exclusion to determine viability and cell density. Monocytes were centrifuged as above and resuspended to approximately $1x10^7$ cells/ml in 37°C HBSS w/out red. Calcein-AM (Molecular Probes; Eugene, OR) was added to 5μM, mixed gently, and incubated 15 minutes in a 37°C water bath, inverting to mix one time during incubation. Monocytes were centrifuged as above, resuspended in 30 mls HBSS w/out red, cell counts determined by Trypan Blue exclusion, and centrifuged again as above to wash.

Monocytes were then again suspended to 2.86×10^6 cells/ml in ice cold RPMI 1640 media, 5% FBS, and placed on ice. FALCON HTS 3 μ Fluoroblok inserts (Becton Dickinson Labware; Franklin Lakes, NJ) were removed from package carefully and placed in FALCON multiwell cell culture insert companion 24 well plates (Becton Dickinson Labware; Franklin Lakes, NJ) using sterile forceps. 350 μls

(approximately 1 x 10^6 cells) of Calcein-AM labeled monocytes were added carefully to each Fluoroblok insert sitting in companion plate, then 900 µl of test sample was carefully pipetted into each companion plate well.

Readings were taken every 30 minutes beginning at time zero on a Cytofluor 2 (PerSeptive Biosystems; Framingham MA), excitation 485 nm, emission 530 nm, and gain at 50. Mean and standard deviation were calculated, then results plotted using Deltagraph 4.0 (SPSS; Chicago, IL). Hu MCP-1, Hu MIP1α, and SDF1α chemokines were obtained from Pharmingen; San Diego, CA.

Results are shown in Figure 1. These data show that the semaphorin A39R inhibits both spontaneous migration of monocytes, as well as monocyte migration towards the monocyte-attracting chemokine MCP-1 (as shown in lines -▲- and -◆-). These data also show that VESPR mediates this inhibitory effect of the semaphorin. M460 is a blocking mAb directed against VESPR. According to these results, M460 partially reverses the inhibitory effects of A39R (see line -Δ-). Further confirming the role of VESPR is the data showing that M461, a non-blocking mAb against VESPR and having the same isotype as M460, does not influence the inhibitory effect of the semaphorin (see line -◊-).

B. EXAMPLE 2: SEMAPHORINS INFLUENCE IMMUNE CELL CHEMOTAXIS AS SHOWN BY UNDER AGAROSE MIGRATION ASSAY

RPMI 1640 was made up to a 2x concentration with FBS (Gibco BRL; Rockville, MD) added to 10% of the final volume, and placed at 55 °C for 15 minutes. Autoclaved 2% Seakem Agarose (FMC Bioproducts; Rockland, Maine) in water was heated until completely liquified by microwaving then placed at 55°C for 15 minutes. Agarose and media were mixed carefully 1:1 to avoid excess air bubbles in a FALCON 50-ml conical tube (Becton Dickinson Labware; Franklin Lakes, NJ) sitting in a beaker of 55°C water. 10 mls of agarose media mix was added to FALCON 10-cm petri dishes (Becton Dickinson Labware; Franklin Lakes, NJ) placed on a level surface and allowed to solidify for 5 minutes.

Plates were then transferred to a 4°C cooler to further harden for 10 minutes. Three wells in a straight line were cut into the hardened agarose with a 3/32-inch steel hole punch approximately 2.4 mm apart, by placing a template below the dish. Three sets of wells were cut per plate in an equidistant radial pattern. Agarose plugs were carefully removed from the wells by aspiration. Human monocytes from apparently healthy donors were separated by counter current centrifugation (elutriation) from freshly drawn leukophoresis packs using a Beckman J-6M/E centrifuge (Beckman Instruments, Inc.; Palo Alto, CA) and aliquots stored under Liquid Nitrogen.

Monocyte samples were thawed rapidly at 37°C and added to 30 ml of ice cold RPMI 1640, 5% FBS in a FALCON 50-ml conical tube then centrifuged at 1400 rpm, 5 minutes, at room temperature. Monocytes were resuspended in 10-mls of above media, placed on ice, and cell count determined by Trypan Blue (Gibco BRL; Rockville, MD) exclusion. Centrifuge monocytes as above and resuspended to 2.0 x 108 cells/ml, keeping cells on ice. Test samples made up in above media in FALCON 15-ML conical tubes and placed on ice.

5 µl of cell suspension were added to center well, 5µl of test sample were added to outer well, and 5 µl of media were added to inner well for each of the three sets of wells per plate. Plates were incubated at 37°C, 5% CO2, 100% methanol for either 30 minutes, or overnight at 4°C. Methanol was aspirated from plate, and 5 ml 4% formalin (Sigma;) added for 30 minutes at room temperature to fix cells. Agarose layer was then carefully removed from plate, and plates stained with crystal violet(;) for 1 minute, crystal violet was removed, plates washed in water and allowed to dry.

Results are shown in Figure 2. These data show the results of the under agarose assays, indicating that migration of immune cells, specifically APCs, in the presence of semaphorin, is inhibited. The bottom frame shows diffuse cells in assay lacking chemokines; the middle frame shows that, in the presence of A39R and MCP1, the cells do not migrate toward the chemo-attractant MCP1, while in the top frame, cells are clearly drawn towards MCP1 in the absence of the semaphorin A39R.

C. Example 3: Semaphorins Influence Immune Cell Chemotaxis and Do Not Induce Cell Death As Shown by Alamar Blue Proliferation Assay

To confirm that the VESPR-mediated semaphorin effect on the monocytes was in fact inhibitory, and not lethal, the following cell proliferation assay was performed:

Cells were prepared for assay by harvesting as appropriate into FALCON 15 ml or 50 ml conical tubes (Becton Dickinson Labware; Franklin Lakes, NJ), then centrifuging at 1200-1500 rpm, room temperature for 5 minutes. Cells were resuspended in media minus growth factor, then centrifuged again and resuspended at density for assay. One ml of cell suspension was added per well of a Falcon 24 well non-tissue culture treated plate (Becton Dickinson Labware; Franklin Lakes, NJ). Test samples were added to cells plus samples at 37°C, 100% humidity, 5% CO2 ranging from 18-72 hours, to 10% of final volume. Readings of Alamar Blue processing were then taken at various time points post addition with a Cytofluor 2 fluorescent plate reader (PerSeptive Biosystems; Framingham, MA) excitation 530 nm, emission 590 nm, and gain at 40. Results were graphed using Deltagraph 4.0 (SPSS Inc.; Chicago, IL). Viability of cell samples over the various incubation periods was tested by trypan blue exclusion indicating A39R had little or no effect on cell viability.

Human monocytes purified by elutriation from freshly drawn leukophoresis packs. TF-1, human bone marrow erythroleukemia cell line were tested in this assay.

Data from the cell proliferation assay are shown in Figure 3. These data show that semaphorins, or semaphorin-receptor complexes influence immune cell motility, but are not lethal to the cells. An increase in fluorescence over time shows that the cells are viable. Cells exposed to A39R remain viable (*see* lines -•-, -□-, -○-, and -◇-).These data confirm that, in cases such as where monocyte migration is inhibited, this immobilization is not the result of cell death.

D. Example 4: Semaphorins Influence Langerhans Cell Migration *In Vivo*

In vivo analysis of the effects of A39R on Langerhans cell migration was performed, to confirm semaphorin effect on cell migration *in vivo*. To begin, mice were anesthetized with 0.3-ml of Avertin(;)

per mouse by intraperitoneal injection. The forearms of each mouse were then shaved, and various amounts of protein or Saline (Baxter Healthcare Corp.; Deerfield, IL) in a 10µl volume were injected by subcutaneous administration using a 1 ml Hamilton syringe (Hamilton Company; Reno, NV) in the front of each forearm.

10-20µl of either a 1% w/v Fluorescein Iso-Thio-Cyanate (FITC) (Sigma; St. Louis, MO) solution made up in a 1:1 Acetone: Dibutylphthalate (Sigma; St. Louis, MO), or 1:1 Acetone: Dibutylphthalate were then "painted" over the injection site in a drop-wise fashion using a P20 pippetteman (Rainin Instrument Company Inc.; Woburn, MA). The mice were allowed to rest 24 hours and then sacrificed by CO2 asphyxiation.

The axillary and brachial lymph nodes from each animal were taken and placed in 20 mls of RPMI 1640 (Gibco BRL; Rockville, MD), 5% FBS (Gibco BRL; Rockville, MD) in a FALCON 50 ml conical tube (Becton Dickinson Labware; Franklin Lakes, NJ) sitting on ice. The conicals were then centrifuged at 1200 rpm for 5 minutes at room temperature, and the supernatant was removed by aspiration. The lymph nodes were resuspended in 10 mls of 200 units/ml Collagenase type 3 (Worthington Biochemical Corp.; Freehold, NJ) made up in Hanks Balanced Salt Solution with calcium and magnesium (Gibco BRL; Rockville, MD), containing 1 g NaCl, pH adjusted to 7.4 with NaOH. Suspensions were then added to FALCON petri dishes (Becton Dickinson Labware; Franklin Lakes, NJ).

Lymph nodes were then gently teased apart with sterile forceps, and incubated at 37°C, 5% CO2, 100% humidity for 30 minutes. Each lymph node suspensions were removed from dish and filtered through a FALCON nylon cell strainer (Becton Dickinson Labware; Franklin Lakes, NJ) sitting in a FALCON 50 ml conical tube on ice. The black rubber end of a plunger from a 5-ml syringe (Becton Dickinson Labware; Franklin Lakes, NJ) was used to mash any large chunks sitting in cell strainer. 10 ml of Hanks Balanced Salt Solution with calcium and magnesium (Gibco BRL; Rockville, MD), containing 1 g NaCl, and pH adjusted to 7.4 with NaOH was added to each dish to wash. Dishes were gently scraped with a COSTAR cell scraper (Corning Inc.; Corning, NY), wash was removed from each plate and added to corresponding conical tube through cell strainer. Lymph node suspension were centrifuged at 1200 rpm, for 5 minutes at room temperature, resuspended in 10 ml of Hanks Balanced Salt Solution without calcium and magnesium (Gibco BRL; Rockville, MD), containing 10 mM EDTA, pH adjusted to 7.4 with HCL, and centrifuged again.

Nycodenz solution stock was made up by adding 15.275 g Nycodenz powder (Gibco BRL; Rockville, MD) to 50 ml, 37°C Baxter water (Baxter Healthcare Corp.; Deerfield, IL), then filtered through a Corning 0.45µ cellulose acetate low protein binding membrane (Corning Inc.; Corning, NY), stock stored at 4°C in the dark.

Nycodenz buffer was made up by adding 9g NaCl, 0.6055 g Tris, 0.22368g Kcl, 0.11167g EDTA, to 1 L of Baxter water, pH adjusted to 7.4, and volume brought up to 1.120L with Baxter water. Nycodenz working solution was made up by adding 46-ml of Nycodenz solution stock to 54-ml Nycodenz buffer.

Cell pellets were resuspended in 5 ml of Nycodenz working solution and removed to FALCON 15-ml conical tubes (Becton Dickinson Labware; Franklin Lakes, NJ). 3ml of Hanks Balanced Salt Solution without calcium and magnesium (Gibco BRL; Rockville, MD), containing 10 mM EDTA, pH adjusted to 7.4 with HCL, were then carefully overlaid on the Nycodenz working solution cell suspensions, then centrifuged at 2700 rpm for 20 minutes at 4°C with the brake off. The interfaces from each resulting gradient were then carefully removed to a FALCON 15ml conical tube by pipetting, and centrifuged at 1200 rpm for 5 minutes at room temperature. Pellets were resuspended in 5 ml of ice cold RPMI 1640, 5% FBS and placed on ice.

Following isolation, cells were stained with a conjugated (phycoerythrin) anti MHC class II stain, and analyzed on a flow cytometer (Becton-Dickinson). Cells that showed double positive staining (*i.e.* positive for FITC and positive for MHC class II) were presumed to be Langerhans cells. Under these conditions, a two fold reduction in the amount of migrating Langerhans cells in animals administered A39R compared to those administered control protein was observed. Specifically, as shown in Figure 4A and Figure 4B, Langerhans cell migration to regional lymph nodes (upper right quadrant) in A39R-treated animals and control animals indicated that, *in vivo*, APC migration to regional lymph nodes was inhibited by the semaphorin (9.19% versus 6.21%).

E. EXAMPLE 5: VESPR IS EXPRESSED AT HIGH LEVELS IN MAST CELLS

Immunohistochemistry (IHC) and standard histological examination of rodent tissues was used to detect the semaphorin binding protein VESPR. Mast cells expressing VESPR bound biotinylated A39R throughout the tissues examined, including spleen, lymph node, sinus, retina, skin and lung. The finding that mast cells express significant amounts of VESPR indicates that interaction with the VESPR ligand, in other words with semaphorins or VESPR agonists or antagonists, will affect the migration of mast cells.

REFERENCES

Baum, J. *et. al.*, J. *Lab. Clin. Med.* 77:501 (1971);
Boyden Chamber, Boyden S.J. *J Exp Med* 115:453
Dodd and Schuchardt, *Cell*, 81:471-74, 1995
Gallin, J.I., *et. al., J. Immunol* 110:223
Giessmann, R., *et al., JI* 162:4567
Goodman *et al.*, U.S. Patent No. 5,935,865, August 10, 1999
Holloway, WO 99/32622, 1999
Inagaki and Furuyama, EP 960,888, 1999
Katoh, N., *Clin. Exp. Immunol.* 108:302
Kayoko, S., *et al., JI* 161:6835
Keller, H.U. *et. al., J. Immunol. Meth.* 1:165,
Kimura and Kikushi, EP 945,505, 1999

Kimura and Kikushi, EP 960,937, 1999

Ma, J. *et al.*, *Cell Immunology* 158:389

Manickasingham, S., *Clin. Exp. Immunol.* 106:304

Michalovich, *et al.*, EP 933,425, 1999

Nelson, R.D. *et. al. JI* 115:1650.

Snyderman, R. *et. al.*, *J. Immunol.* 108:857,

Tessier-Lavigne, M and Goodman C.S. Science 1996, 274:1123.

Wang, B. *et al.*, *JI* 162:277

Wang, B., *et al.*, *Immunology* 88:284

Wang, B., *et al.*, *JI* 159:6148

Woods, G.M., *Immunology* 88:134

Zigmond, S.H. *et. al.*, *J. Exp. Med* 137:187

CLAIMS

1. Use of a semaphorin or an agonistic antibody to a VESPR in the manufacture of a medicament for neoplastic disease; auto-immune or hypersensitivity disorders characterized in part by an inflammatory response; promotion or suppression of angiogenesis; or food intolerance.

2. Use of a VESPR in the manufacture of a medicament for neoplastic disease; auto-immune or hypersensitivity disorders characterized in part by an inflammatory response; promotion or suppression of angiogenesis; or food intolerance.

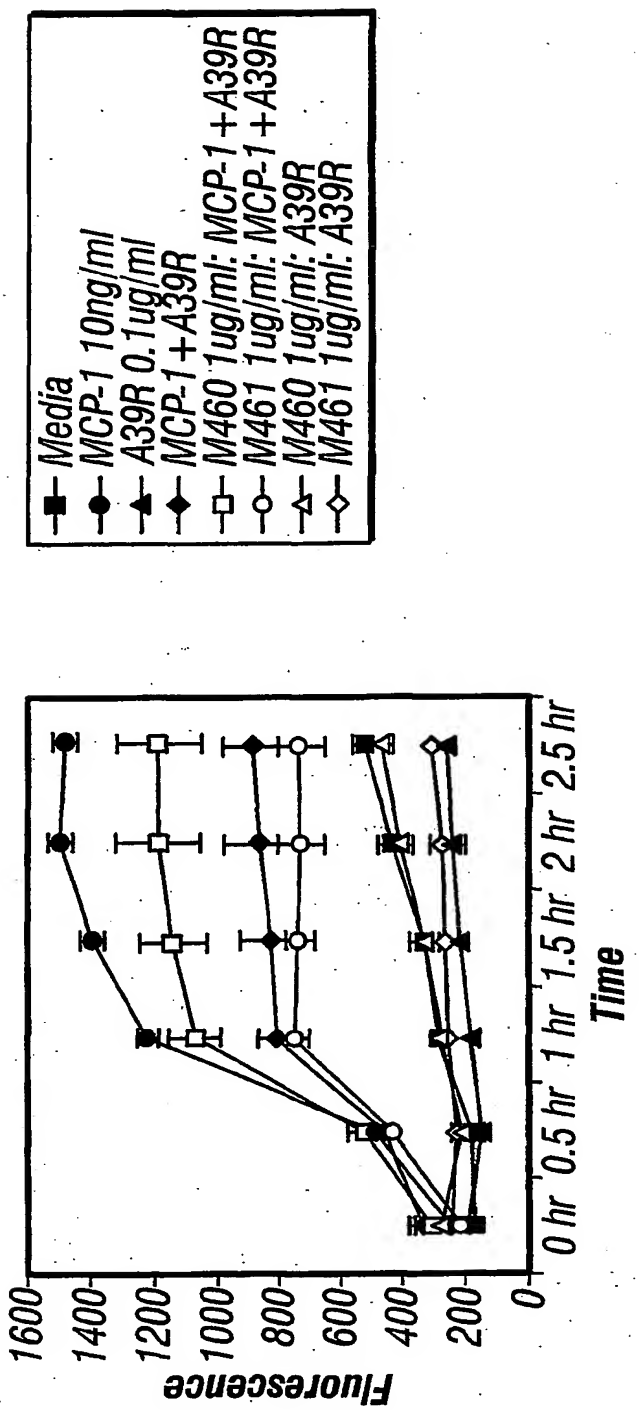
Media
MCP-1 10ng/ml
A39R 0.1ug/ml
MCP-1+A39R
M460 1ug/ml: MCP-1+A39R
M461 1ug/ml: MCP-1+A39R
M460 1ug/ml: A39R
M461 1ug/ml: A39R
Fluorescence
1600
1400
1200
1000
800
600
400
200
0
0 hr
0.5 hr
1 hr
1.5 hr
2 hr
2.5 hr
Time

FIG. 1

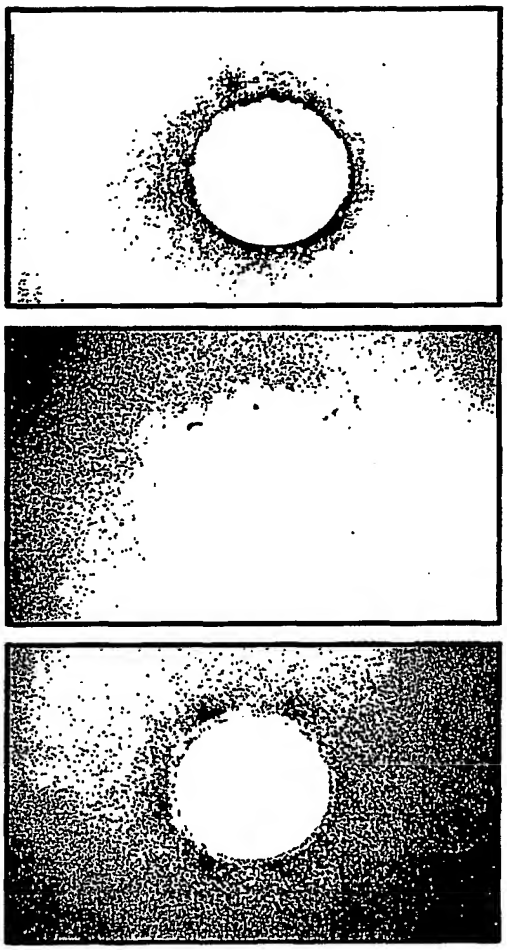

FIG. 2

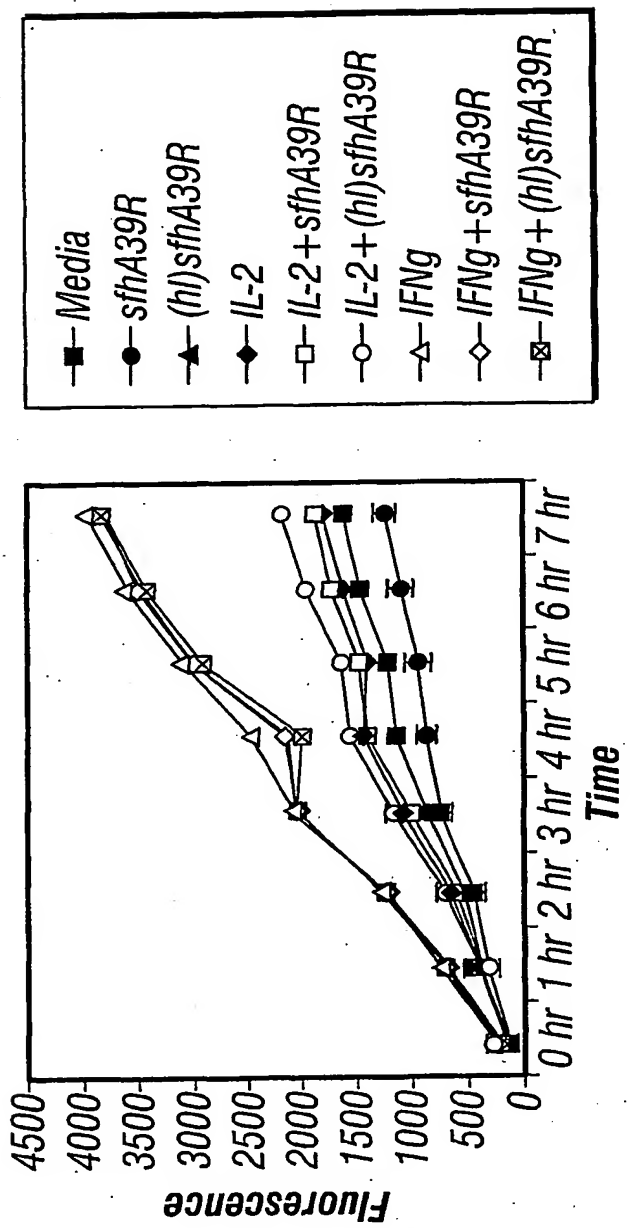
Media
sfhA39R
(hl)sfhA39R
IL-2
IL-2+sfhA39R
IL-2+(hl)sfhA39R
IFNg
IFNg+sfhA39R
IFNg+(hl)sfhA39R
Fluorescence
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0 hr
1 hr
2 hr
3 hr
4 hr
5 hr
6 hr
7 hr
Time

FIG. 3

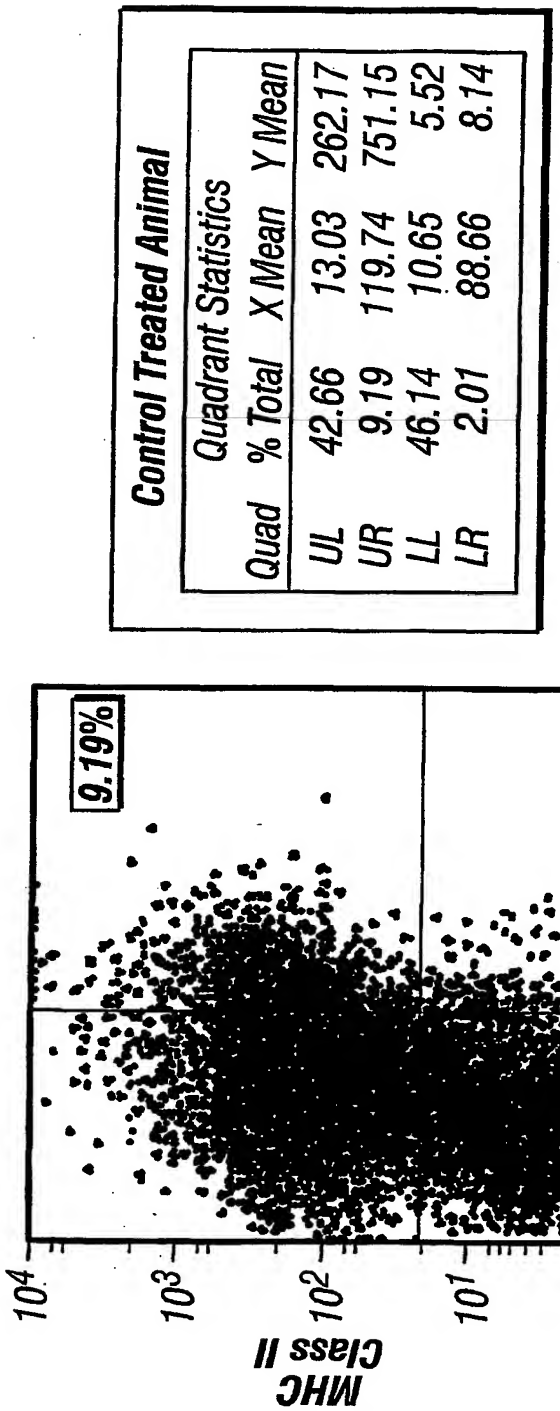
Control Treated Animal
Quadrant Statistics
Quad % Total X Mean Y Mean
UL 42.66 13.03 262.17
UR 9.19 119.74 751.15
LL 46.14 10.65 5.52
LR 2.01 88.66 8.14
9.19%
MHC Class II
FITC
10^0
10^1
10^2
10^3
10^4

FIG. 4A

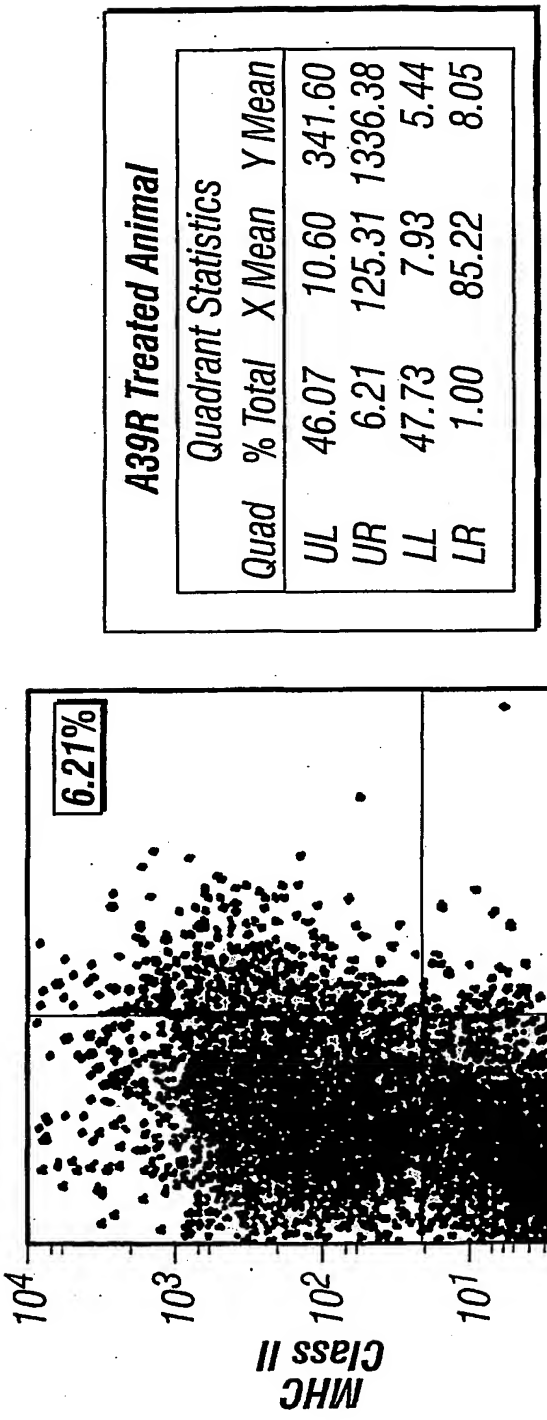
A39R Treated Animal
Quadrant Statistics
Quad % Total X Mean Y Mean
UL 46.07 10.60 341.60
UR 6.21 125.31 1336.38
LL 47.73 7.93 5.44
LR 1.00 85.22 8.05
6.21%
MHC Class II
FITC
10^0
10^1
10^2
10^3
10^4

FIG. 4B

INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4707 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..4707

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATG GAG GTC TCC CGG AGG AAG GCG CCG CCG CGC CCC CCG CGC CCC GCA      48
Met Glu Val Ser Arg Arg Lys Ala Pro Pro Arg Pro Pro Arg Pro Ala
 1               5                  10                  15

GCG CCA CTG CCC CTG CTC GCC TAT CTG CTG GCA CTG GCG GCT CCC GGC      96
Ala Pro Leu Pro Leu Leu Ala Tyr Leu Leu Ala Leu Ala Ala Pro Gly
            20                  25                  30

CGG GGC GCG GAC GAG CCC GTG TGG CGG TCG GAG CAA GCC ATC GGA GCC     144
Arg Gly Ala Asp Glu Pro Val Trp Arg Ser Glu Gln Ala Ile Gly Ala
        35                  40                  45

ATC GCG GCG AGC CAG GAG GAC GGC GTG TTT GTG GCG AGC GGC AGC TGC     192
Ile Ala Ala Ser Gln Glu Asp Gly Val Phe Val Ala Ser Gly Ser Cys
    50                  55                  60

CTG GAC CAG CTG GAC TAC AGC CTG GAG CAC AGC CTC TCG CGC CTG TAC     240
Leu Asp Gln Leu Asp Tyr Ser Leu Glu His Ser Leu Ser Arg Leu Tyr
65                  70                  75                  80

CGG GAC CAA GCG GGC AAC TGC ACA GAG CCG GTC TCG CTG GCG CCC CCC     288
Arg Asp Gln Ala Gly Asn Cys Thr Glu Pro Val Ser Leu Ala Pro Pro
                85                  90                  95

GCG CGG CCC CGG CCC GGG AGC AGC TTC AGC AAG CTG CTG CTG CCC TAC     336
Ala Arg Pro Arg Pro Gly Ser Ser Phe Ser Lys Leu Leu Leu Pro Tyr
            100                 105                 110

CGC GAG GGG GCG GCC GGC CTC GGG GGG CTG CTG CTC ACC GGC TGG ACC     384
Arg Glu Gly Ala Ala Gly Leu Gly Gly Leu Leu Leu Thr Gly Trp Thr
        115                 120                 125
```

```
TTC GAC CGG GGC GCC TGC GAG GTG CGG CCC CTG GGC AAC CTG AGC CGC        432
Phe Asp Arg Gly Ala Cys Glu Val Arg Pro Leu Gly Asn Leu Ser Arg
    130                 135                 140

AAC TCC CTG CGC AAC GGC ACC GAG GTG GTG TCG TGC CAC CCG CAG GGC        480
Asn Ser Leu Arg Asn Gly Thr Glu Val Val Ser Cys His Pro Gln Gly
145                 150                 155                 160

TCG ACG GCC GGC GTG GTG TAC CGC GCG GGC CGG AAC AAC CGC TGG TAC        528
Ser Thr Ala Gly Val Val Tyr Arg Ala Gly Arg Asn Asn Arg Trp Tyr
                165                 170                 175

CTG GCG GTG GCC GCC ACC TAC GTG CTG CCT GAG CCG GAG ACG GCG AGC        576
Leu Ala Val Ala Ala Thr Tyr Val Leu Pro Glu Pro Glu Thr Ala Ser
            180                 185                 190

CGC TGC AAC CCC GCG GCA TCC GAC CAC GAC ACG GCC ATC GCG CTC AAG        624
Arg Cys Asn Pro Ala Ala Ser Asp His Asp Thr Ala Ile Ala Leu Lys
        195                 200                 205

GAC ACG GAG GGG CGC AGC CTG GCC ACG CAG GAG CTG GGG CGC CTC AAG        672
Asp Thr Glu Gly Arg Ser Leu Ala Thr Gln Glu Leu Gly Arg Leu Lys
    210                 215                 220

CTG TGC GAG GGC GCG GGC AGC CTG CAC TTC GTG GAC GCC TTT CTC TGG        720
Leu Cys Glu Gly Ala Gly Ser Leu His Phe Val Asp Ala Phe Leu Trp
225                 230                 235                 240

AAC GGC AGC ATC TAC TTC CCC TAC TAC CCC TAC AAC TAT ACG AGC GGC        768
Asn Gly Ser Ile Tyr Phe Pro Tyr Tyr Pro Tyr Asn Tyr Thr Ser Gly
                245                 250                 255

GCT GCC ACC GGC TGG CCC AGC ATG GCG CGC ATC GCG CAG AGC ACC GAG        816
Ala Ala Thr Gly Trp Pro Ser Met Ala Arg Ile Ala Gln Ser Thr Glu
            260                 265                 270

GTG CTG TTC CAG GGC CAG GCA TCC CTC GAC TGC GGC CAC GGC CAC CCC        864
Val Leu Phe Gln Gly Gln Ala Ser Leu Asp Cys Gly His Gly His Pro
        275                 280                 285

GAC GGC CGC CGC CTG CTC CTC TCC TCC AGC CTA GTG GAG GCC CTG GAC        912
Asp Gly Arg Arg Leu Leu Leu Ser Ser Ser Leu Val Glu Ala Leu Asp
    290                 295                 300

GTC TGG GCG GGA GTG TTC AGC GCG GCC GCT GGA GAG GGC CAG GAG CGG        960
Val Trp Ala Gly Val Phe Ser Ala Ala Ala Gly Glu Gly Gln Glu Arg
305                 310                 315                 320

CGC TCC CCC ACC ACC ACG GCG CTC TGC CTC TTC AGA ATG AGT GAG ATC       1008
Arg Ser Pro Thr Thr Thr Ala Leu Cys Leu Phe Arg Met Ser Glu Ile
                325                 330                 335
```

```
CAG GCG CGC GCC AAG AGG GTC AGC TGG GAC TTC AAG ACG GCC GAG AGC      1056
Gln Ala Arg Ala Lys Arg Val Ser Trp Asp Phe Lys Thr Ala Glu Ser
            340                 345                 350

CAC TGC AAA GAA GGG GAT CAA CCT GAA AGA GTC CAA CCA ATC GCA TCA      1104
His Cys Lys Glu Gly Asp Gln Pro Glu Arg Val Gln Pro Ile Ala Ser
        355                 360                 365

TCT ACC TTG ATC CAT TCC GAC CTG ACA TCC GTT TAT GGC ACC GTG GTA      1152
Ser Thr Leu Ile His Ser Asp Leu Thr Ser Val Tyr Gly Thr Val Val
    370                 375                 380

ATG AAC AGG ACT GTT TTA TTC TTG GGG ACT GGA GAT GGC CAG TTA CTT      1200
Met Asn Arg Thr Val Leu Phe Leu Gly Thr Gly Asp Gly Gln Leu Leu
385                 390                 395                 400

AAG GTT ATT CTT GGT GAG AAT TTG ACT TCA AAT TGT CCA GAG GTT ATC      1248
Lys Val Ile Leu Gly Glu Asn Leu Thr Ser Asn Cys Pro Glu Val Ile
                405                 410                 415

TAT GAA ATT AAA GAA GAG ACA CCT GTT TTC TAC AAA CTC GTT CCT GAT      1296
Tyr Glu Ile Lys Glu Glu Thr Pro Val Phe Tyr Lys Leu Val Pro Asp
            420                 425                 430

CCT GTG AAG AAT ATC TAC ATT TAT CTA ACA GCT GGG AAA GAG GTG AGG      1344
Pro Val Lys Asn Ile Tyr Ile Tyr Leu Thr Ala Gly Lys Glu Val Arg
        435                 440                 445

AGA ATT CGT GTT GCA AAC TGC AAT AAA CAT AAA TCC TGT TCG GAG TGT      1392
Arg Ile Arg Val Ala Asn Cys Asn Lys His Lys Ser Cys Ser Glu Cys
    450                 455                 460

TTA ACA GCC ACA GAC CCT CAC TGC GGT TGG TGC CAT TCG CTA CAA AGG      1440
Leu Thr Ala Thr Asp Pro His Cys Gly Trp Cys His Ser Leu Gln Arg
465                 470                 475                 480

TGC ACT TTT CAA GGA GAT TGT GTA CAT TCA GAG AAC TTA GAA AAC TGG      1488
Cys Thr Phe Gln Gly Asp Cys Val His Ser Glu Asn Leu Glu Asn Trp
                485                 490                 495

CTG GAT ATT TCG TCT GGA GCA AAA AAG TGC CCT AAA ATT CAG ATA ATT      1536
Leu Asp Ile Ser Ser Gly Ala Lys Lys Cys Pro Lys Ile Gln Ile Ile
            500                 505                 510

CGA AGC AGT AAA GAA AAG ACT ACA GTG ACT ATG GTG GGA AGC TTC TCT      1584
Arg Ser Ser Lys Glu Lys Thr Thr Val Thr Met Val Gly Ser Phe Ser
        515                 520                 525

CCA AGA CAC TCA AAG TGC ATG GTG AAG AAT GTG GAC TCT AGC AGG GAG      1632
Pro Arg His Ser Lys Cys Met Val Lys Asn Val Asp Ser Ser Arg Glu
    530                 535                 540

CTC TGC CAG AAT AAA AGT CAG CCC AAC CGG ACC TGC ACC TGT AGC ATC      1680
```

```
Leu Cys Gln Asn Lys Ser Gln Pro Asn Arg Thr Cys Thr Cys Ser Ile
545                 550                 555                 560

CCA ACC AGA GCA ACC TAC AAA GAT GTT TCA GTT GTC AAC GTG ATG TTC      1728
Pro Thr Arg Ala Thr Tyr Lys Asp Val Ser Val Val Asn Val Met Phe
                565                 570                 575

TCC TTC GGT TCT TGG AAT TTA TCA GAC AGA TTC AAC TTT ACC AAC TGC      1776
Ser Phe Gly Ser Trp Asn Leu Ser Asp Arg Phe Asn Phe Thr Asn Cys
            580                 585                 590

TCA TCA TTA AAA GAA TGC CCA GCA TGC GTA GAA ACT GGC TGC GCG TGG      1824
Ser Ser Leu Lys Glu Cys Pro Ala Cys Val Glu Thr Gly Cys Ala Trp
        595                 600                 605

TGT AAA AGT GCA AGA AGG TGT ATC CAC CCC TTC ACA GCT TGC GAC CCT      1872
Cys Lys Ser Ala Arg Arg Cys Ile His Pro Phe Thr Ala Cys Asp Pro
    610                 615                 620

TCT GAT TAT GAG AGA AAC CAG GAA CAG TGT CCA GTG GCT GTC GAG AAG      1920
Ser Asp Tyr Glu Arg Asn Gln Glu Gln Cys Pro Val Ala Val Glu Lys
625                 630                 635                 640

ACA TCA GGA GGA GGA AGA CCC AAG GAG AAC AAG GGG AAC AGA ACC AAC      1968
Thr Ser Gly Gly Gly Arg Pro Lys Glu Asn Lys Gly Asn Arg Thr Asn
                645                 650                 655

CAG GCT TTA CAG GTC TTC TAC ATT AAG TCC ATT GAG CCA CAG AAA GTA      2016
Gln Ala Leu Gln Val Phe Tyr Ile Lys Ser Ile Glu Pro Gln Lys Val
            660                 665                 670

TCG ACA TTA GGG AAA AGC AAC GTG ATA GTA ACG GGA GCA AAC TTT ACC      2064
Ser Thr Leu Gly Lys Ser Asn Val Ile Val Thr Gly Ala Asn Phe Thr
        675                 680                 685

CGG GCA TCG AAC ATC ACA ATG ATC CTG AAA GGA ACC AGT ACC TGT GAT      2112
Arg Ala Ser Asn Ile Thr Met Ile Leu Lys Gly Thr Ser Thr Cys Asp
    690                 695                 700

AAG GAT GTG ATA CAG GTT AGC CAT GTG CTA AAT GAC ACC CAC ATG AAA      2160
Lys Asp Val Ile Gln Val Ser His Val Leu Asn Asp Thr His Met Lys
705                 710                 715                 720

TTC TCT CTT CCA TCA AGC CGG AAA GAA ATG AAG GAT GTG TGT ATC CAG      2208
Phe Ser Leu Pro Ser Ser Arg Lys Glu Met Lys Asp Val Cys Ile Gln
                725                 730                 735

TTT GAT GGT GGG AAC TGC TCT TCT GTG GGA TCC TTA TCC TAC ATT GCT      2256
Phe Asp Gly Gly Asn Cys Ser Ser Val Gly Ser Leu Ser Tyr Ile Ala
            740                 745                 750

CTG CCA CAT TGT TCC CTT ATA TTT CCT GCT ACC ACC TGG ATC AGT GGT      2304
Leu Pro His Cys Ser Leu Ile Phe Pro Ala Thr Thr Trp Ile Ser Gly
```

```
        755                 760                 765

GGT CAA AAT ATA ACC ATG ATG GGC AGA AAT TTT GAT GTA ATT GAC AAC      2352
Gly Gln Asn Ile Thr Met Met Gly Arg Asn Phe Asp Val Ile Asp Asn
    770                 775                 780

TTA ATC ATT TCA CAT GAA TTA AAA GGA AAC ATA AAT GTC TCT GAA TAT      2400
Leu Ile Ile Ser His Glu Leu Lys Gly Asn Ile Asn Val Ser Glu Tyr
785                 790                 795                 800

TGT GTG GCG ACT TAC TGC GGG TTT TTA GCC CCC AGT TTA AAG AGT TCA      2448
Cys Val Ala Thr Tyr Cys Gly Phe Leu Ala Pro Ser Leu Lys Ser Ser
                805                 810                 815

AAA GTG CGC ACG AAT GTC ACT GTG AAG CTG AGA GTA CAA GAC ACC TAC      2496
Lys Val Arg Thr Asn Val Thr Val Lys Leu Arg Val Gln Asp Thr Tyr
            820                 825                 830

TTG GAT TGT GGA ACC CTG CAG TAT CGG GAG GAC CCC AGA TTC ACG GGG      2544
Leu Asp Cys Gly Thr Leu Gln Tyr Arg Glu Asp Pro Arg Phe Thr Gly
        835                 840                 845

TAT CGG GTG GAA TCC GAG GTG GAC ACA GAA CTG GAA GTG AAA ATT CAA      2592
Tyr Arg Val Glu Ser Glu Val Asp Thr Glu Leu Glu Val Lys Ile Gln
    850                 855                 860

AAA GAA AAT GAC AAC TTC AAT ATT TCC AAA AAA GAC ATT GAA ATT ACT      2640
Lys Glu Asn Asp Asn Phe Asn Ile Ser Lys Lys Asp Ile Glu Ile Thr
865                 870                 875                 880

CTC TTC CAT GGG GAA AAT GGG CAA TTA AAT TGC AGT TTT GAA AAT ATT      2688
Leu Phe His Gly Glu Asn Gly Gln Leu Asn Cys Ser Phe Glu Asn Ile
                885                 890                 895

ACT AGA AAT CAA GAT CTT ACC ACC ATC CTT TGC AAA ATT AAA GGC ATC      2736
Thr Arg Asn Gln Asp Leu Thr Thr Ile Leu Cys Lys Ile Lys Gly Ile
            900                 905                 910

AAG ACT GCA AGC ACC ATT GCC AAC TCT TCT AAG AAA GTT CGG GTC AAG      2784
Lys Thr Ala Ser Thr Ile Ala Asn Ser Ser Lys Lys Val Arg Val Lys
        915                 920                 925

CTG GGA AAC CTG GAG CTC TAC GTC GAG CAG GAG TCA GTT CCT TCC ACA      2832
Leu Gly Asn Leu Glu Leu Tyr Val Glu Gln Glu Ser Val Pro Ser Thr
    930                 935                 940

TGG TAT TTT CTG ATT GTG CTC CCT GTC TTG CTA GTG ATT GTC ATT TTT      2880
Trp Tyr Phe Leu Ile Val Leu Pro Val Leu Leu Val Ile Val Ile Phe
945                 950                 955                 960

GCG GCC GTG GGG GTG ACC AGG CAC AAA TCG AAG GAG CTG AGT CGC AAA      2928
Ala Ala Val Gly Val Thr Arg His Lys Ser Lys Glu Leu Ser Arg Lys
                965                 970                 975
```

```
CAG AGT CAA CAA CTA GAA TTG CTG GAA AGC GAG CTC CGG AAA GAG ATA      2976
Gln Ser Gln Gln Leu Glu Leu Leu Glu Ser Glu Leu Arg Lys Glu Ile
            980                 985                 990

CGT GAC GGC TTT GCT GAG CTG CAG ATG GAT AAA TTG GAT GTG GTT GAT      3024
Arg Asp Gly Phe Ala Glu Leu Gln Met Asp Lys Leu Asp Val Val Asp
        995                 1000                1005

AGT TTT GGA ACT GTT CCC TTC CTT GAC TAC AAA CAT TTT GCT CTG AGA      3072
Ser Phe Gly Thr Val Pro Phe Leu Asp Tyr Lys His Phe Ala Leu Arg
    1010                1015                1020

ACT TTC TTC CCT GAG TCA GGT GGC TTC ACC CAC ATC TTC ACT GAA GAT      3120
Thr Phe Phe Pro Glu Ser Gly Gly Phe Thr His Ile Phe Thr Glu Asp
1025                1030                1035                1040

ATG CAT AAC AGA GAC GCC AAC GAC AAG AAT GAA AGT CTC ACA GCT TTG      3168
Met His Asn Arg Asp Ala Asn Asp Lys Asn Glu Ser Leu Thr Ala Leu
                1045                1050                1055

GAT GCC CTA ATC TGT AAT AAA AGC TTT CTT GTT ACT GTC ATC CAC ACC      3216
Asp Ala Leu Ile Cys Asn Lys Ser Phe Leu Val Thr Val Ile His Thr
            1060                1065                1070

CTT GAA AAG CAG AAG AAC TTT TCT GTG AAG GAC AGG TGT CTG TTT GCC      3264
Leu Glu Lys Gln Lys Asn Phe Ser Val Lys Asp Arg Cys Leu Phe Ala
        1075                1080                1085

TCC TTC CTA ACC ATT GCA CTG CAA ACC AAG CTG GTC TAC CTG ACC AGC      3312
Ser Phe Leu Thr Ile Ala Leu Gln Thr Lys Leu Val Tyr Leu Thr Ser
    1090                1095                1100

ATC CTA GAG GTG CTG ACC AGG GAC TTG ATG GAA CAG TGT AGT AAC ATG      3360
Ile Leu Glu Val Leu Thr Arg Asp Leu Met Glu Gln Cys Ser Asn Met
1105                1110                1115                1120

CAG CCG AAA CTC ATG CTG AGA CGC ACG GAG TCC GTC GTC GAA AAA CTC      3408
Gln Pro Lys Leu Met Leu Arg Arg Thr Glu Ser Val Val Glu Lys Leu
                1125                1130                1135

CTC ACA AAC TGG ATG TCC GTC TGC CTT TCT GGA TTT CTC CGG GAG ACT      3456
Leu Thr Asn Trp Met Ser Val Cys Leu Ser Gly Phe Leu Arg Glu Thr
            1140                1145                1150

GTC GGA GAG CCC TTC TAT TTG CTG GTG ACG ACT CTG AAC CAG AAA ATT      3504
Val Gly Glu Pro Phe Tyr Leu Leu Val Thr Thr Leu Asn Gln Lys Ile
        1155                1160                1165

AAC AAG GGT CCC GTG GAT GTA ATC ACT TGC AAA GCC CTG TAC ACA CTT      3552
Asn Lys Gly Pro Val Asp Val Ile Thr Cys Lys Ala Leu Tyr Thr Leu
    1170                1175                1180
```

```
AAT GAA GAC TGG CTG TTG TGG CAG GTT CCG GAA TTC AGT ACT GTG GCA      3600
Asn Glu Asp Trp Leu Leu Trp Gln Val Pro Glu Phe Ser Thr Val Ala
1185                1190                1195                1200

TTA AAC GTC GTC TTT GAA AAA ATC CCG GAA AAC GAG AGT GCA GAT GTC      3648
Leu Asn Val Val Phe Glu Lys Ile Pro Glu Asn Glu Ser Ala Asp Val
                1205                1210                1215

TGT CGG AAT ATT TCA GTC AAT GTT CTC GAC TGT GAC ACC ATT GGC CAA      3696
Cys Arg Asn Ile Ser Val Asn Val Leu Asp Cys Asp Thr Ile Gly Gln
            1220                1225                1230

GCC AAA GAA AAG ATT TTC CAA GCA TTC TTA AGC AAA AAT GGC TCT CCT      3744
Ala Lys Glu Lys Ile Phe Gln Ala Phe Leu Ser Lys Asn Gly Ser Pro
        1235                1240                1245

TAT GGA CTT CAG CTT AAT GAA ATT GGT CTT GAG CTT CAA ATG GGC ACA      3792
Tyr Gly Leu Gln Leu Asn Glu Ile Gly Leu Glu Leu Gln Met Gly Thr
    1250                1255                1260

CGA CAG AAA GAA CTT CTG GAC ATC GAC AGT TCC TCC GTG ATT CTT GAA      3840
Arg Gln Lys Glu Leu Leu Asp Ile Asp Ser Ser Ser Val Ile Leu Glu
1265                1270                1275                1280

GAT GGA ATC ACC AAG CTA AAC ACC ATT GGC CAC TAT GAG ATA TCA AAT      3888
Asp Gly Ile Thr Lys Leu Asn Thr Ile Gly His Tyr Glu Ile Ser Asn
                1285                1290                1295

GGA TCC ACT ATA AAA GTC TTT AAG AAG ATA GCA AAT TTT ACT TCA GAT      3936
Gly Ser Thr Ile Lys Val Phe Lys Lys Ile Ala Asn Phe Thr Ser Asp
            1300                1305                1310

GTG GAG TAC TCG GAT GAC CAC TGC CAT TTG ATT TTA CCA GAT TCG GAA      3984
Val Glu Tyr Ser Asp Asp His Cys His Leu Ile Leu Pro Asp Ser Glu
        1315                1320                1325

GCA TTC CAA GAT GTG CAA GGA AAG AGA CAT CGA GGG AAG CAC AAG TTC      4032
Ala Phe Gln Asp Val Gln Gly Lys Arg His Arg Gly Lys His Lys Phe
    1330                1335                1340

AAA GTA AAA GAA ATG TAT CTG ACA AAG CTG CTG TCG ACC AAG GTG GCA      4080
Lys Val Lys Glu Met Tyr Leu Thr Lys Leu Leu Ser Thr Lys Val Ala
1345                1350                1355                1360

ATT CAT TCT GTG CTT GAA AAA CTT TTT AGA AGC ATT TGG AGT TTA CCC      4128
Ile His Ser Val Leu Glu Lys Leu Phe Arg Ser Ile Trp Ser Leu Pro
                1365                1370                1375

AAC AGC AGA GCT CCA TTT GCT ATA AAA TAC TTT TTT GAC TTT TTG GAC      4176
Asn Ser Arg Ala Pro Phe Ala Ile Lys Tyr Phe Phe Asp Phe Leu Asp
            1380                1385                1390

GCC CAG GCT GAA AAC AAA AAA ATC ACA GAT CCT GAC GTC GTA CAT ATT      4224
```

```
Ala Gln Ala Glu Asn Lys Lys Ile Thr Asp Pro Asp Val Val His Ile
        1395                1400                1405

TGG AAA ACA AAC AGC CTT CCT CTT CGC TTC TGG GTA AAC ATC CTG AAG      4272
Trp Lys Thr Asn Ser Leu Pro Leu Arg Phe Trp Val Asn Ile Leu Lys
    1410                1415                1420

AAC CCT CAG TTT GTC TTT GAC ATT AAG AAG ACA CCA CAT ATA GAC GGC      4320
Asn Pro Gln Phe Val Phe Asp Ile Lys Lys Thr Pro His Ile Asp Gly
1425                1430                1435                1440

TGT TTG TCA GTG ATT GCC CAG GCA TTC ATG GAT GCA TTT TCT CTC ACA      4368
Cys Leu Ser Val Ile Ala Gln Ala Phe Met Asp Ala Phe Ser Leu Thr
                1445                1450                1455

GAG CAG CAA CTA GGG AAG GAA GCA CCA ACT AAT AAG CTT CTC TAT GCC      4416
Glu Gln Gln Leu Gly Lys Glu Ala Pro Thr Asn Lys Leu Leu Tyr Ala
            1460                1465                1470

AAG GAT ATC CCA ACC TAC AAA GAA GAA GTA AAA TCT TAT TAC AAA GCA      4464
Lys Asp Ile Pro Thr Tyr Lys Glu Glu Val Lys Ser Tyr Tyr Lys Ala
        1475                1480                1485

ATC AGG GAT TTG CCT CCA TTG TCA TCC TCA GAA ATG GAA GAA TTT TTA      4512
Ile Arg Asp Leu Pro Pro Leu Ser Ser Ser Glu Met Glu Glu Phe Leu
    1490                1495                1500

ACT CAG GAA TCT AAG AAA CAT GAA AAT GAA TTT AAT GAA GAA GTG GCC      4560
Thr Gln Glu Ser Lys Lys His Glu Asn Glu Phe Asn Glu Glu Val Ala
1505                1510                1515                1520

TTG ACA GAA ATT TAC AAA TAC ATC GTA AAA TAT TTT GAT GAG ATT CTA      4608
Leu Thr Glu Ile Tyr Lys Tyr Ile Val Lys Tyr Phe Asp Glu Ile Leu
                1525                1530                1535

AAT AAA CTA GAA AGA GAA CGA GGG CTG GAA GAA GCT CAG AAA CAA CTC      4656
Asn Lys Leu Glu Arg Glu Arg Gly Leu Glu Glu Ala Gln Lys Gln Leu
            1540                1545                1550

TTG CAT GTA AAA GTC TTA TTT GAT GAA AAG AAG AAA TGC AAG TGG ATG      4704
Leu His Val Lys Val Leu Phe Asp Glu Lys Lys Lys Cys Lys Trp Met
        1555                1560                1565

TAA                                                                 4707
 *
```

INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1569 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Glu Val Ser Arg Arg Lys Ala Pro Pro Arg Pro Pro Arg Pro Ala
 1               5                  10                  15

Ala Pro Leu Pro Leu Leu Ala Tyr Leu Leu Ala Leu Ala Ala Pro Gly
            20                  25                  30

Arg Gly Ala Asp Glu Pro Val Trp Arg Ser Glu Gln Ala Ile Gly Ala
        35                  40                  45

Ile Ala Ala Ser Gln Glu Asp Gly Val Phe Val Ala Ser Gly Ser Cys
    50                  55                  60

Leu Asp Gln Leu Asp Tyr Ser Leu Glu His Ser Leu Ser Arg Leu Tyr
65                  70                  75                  80

Arg Asp Gln Ala Gly Asn Cys Thr Glu Pro Val Ser Leu Ala Pro Pro
                85                  90                  95

Ala Arg Pro Arg Pro Gly Ser Ser Phe Ser Lys Leu Leu Leu Pro Tyr
            100                 105                 110

Arg Glu Gly Ala Ala Gly Leu Gly Gly Leu Leu Leu Thr Gly Trp Thr
        115                 120                 125

Phe Asp Arg Gly Ala Cys Glu Val Arg Pro Leu Gly Asn Leu Ser Arg
    130                 135                 140

Asn Ser Leu Arg Asn Gly Thr Glu Val Val Ser Cys His Pro Gln Gly
145                 150                 155                 160

Ser Thr Ala Gly Val Val Tyr Arg Ala Gly Arg Asn Asn Arg Trp Tyr
                165                 170                 175

Leu Ala Val Ala Ala Thr Tyr Val Leu Pro Glu Pro Glu Thr Ala Ser
            180                 185                 190

Arg Cys Asn Pro Ala Ala Ser Asp His Asp Thr Ala Ile Ala Leu Lys
        195                 200                 205

Asp Thr Glu Gly Arg Ser Leu Ala Thr Gln Glu Leu Gly Arg Leu Lys
    210                 215                 220

Leu Cys Glu Gly Ala Gly Ser Leu His Phe Val Asp Ala Phe Leu Trp
225                 230                 235                 240

Asn Gly Ser Ile Tyr Phe Pro Tyr Tyr Pro Tyr Asn Tyr Thr Ser Gly
                245                 250                 255
```

```
Ala Ala Thr Gly Trp Pro Ser Met Ala Arg Ile Ala Gln Ser Thr Glu
            260                 265                 270

Val Leu Phe Gln Gly Gln Ala Ser Leu Asp Cys Gly His Gly His Pro
        275                 280                 285

Asp Gly Arg Arg Leu Leu Leu Ser Ser Ser Leu Val Glu Ala Leu Asp
    290                 295                 300

Val Trp Ala Gly Val Phe Ser Ala Ala Ala Gly Glu Gly Gln Glu Arg
305                 310                 315                 320

Arg Ser Pro Thr Thr Thr Ala Leu Cys Leu Phe Arg Met Ser Glu Ile
                325                 330                 335

Gln Ala Arg Ala Lys Arg Val Ser Trp Asp Phe Lys Thr Ala Glu Ser
            340                 345                 350

His Cys Lys Glu Gly Asp Gln Pro Glu Arg Val Gln Pro Ile Ala Ser
        355                 360                 365

Ser Thr Leu Ile His Ser Asp Leu Thr Ser Val Tyr Gly Thr Val Val
    370                 375                 380

Met Asn Arg Thr Val Leu Phe Leu Gly Thr Gly Asp Gly Gln Leu Leu
385                 390                 395                 400

Lys Val Ile Leu Gly Glu Asn Leu Thr Ser Asn Cys Pro Glu Val Ile
                405                 410                 415

Tyr Glu Ile Lys Glu Glu Thr Pro Val Phe Tyr Lys Leu Val Pro Asp
            420                 425                 430

Pro Val Lys Asn Ile Tyr Ile Tyr Leu Thr Ala Gly Lys Glu Val Arg
        435                 440                 445

Arg Ile Arg Val Ala Asn Cys Asn Lys His Lys Ser Cys Ser Glu Cys
    450                 455                 460

Leu Thr Ala Thr Asp Pro His Cys Gly Trp Cys His Ser Leu Gln Arg
465                 470                 475                 480

Cys Thr Phe Gln Gly Asp Cys Val His Ser Glu Asn Leu Glu Asn Trp
                485                 490                 495

Leu Asp Ile Ser Ser Gly Ala Lys Lys Cys Pro Lys Ile Gln Ile Ile
            500                 505                 510

Arg Ser Ser Lys Glu Lys Thr Thr Val Thr Met Val Gly Ser Phe Ser
        515                 520                 525

Pro Arg His Ser Lys Cys Met Val Lys Asn Val Asp Ser Ser Arg Glu
    530                 535                 540
```

```
Leu Cys Gln Asn Lys Ser Gln Pro Asn Arg Thr Cys Thr Cys Ser Ile
545                 550                 555                 560

Pro Thr Arg Ala Thr Tyr Lys Asp Val Ser Val Val Asn Val Met Phe
                565                 570                 575

Ser Phe Gly Ser Trp Asn Leu Ser Asp Arg Phe Asn Phe Thr Asn Cys
            580                 585                 590

Ser Ser Leu Lys Glu Cys Pro Ala Cys Val Glu Thr Gly Cys Ala Trp
        595                 600                 605

Cys Lys Ser Ala Arg Arg Cys Ile His Pro Phe Thr Ala Cys Asp Pro
    610                 615                 620

Ser Asp Tyr Glu Arg Asn Gln Glu Gln Cys Pro Val Ala Val Glu Lys
625                 630                 635                 640

Thr Ser Gly Gly Gly Arg Pro Lys Glu Asn Lys Gly Asn Arg Thr Asn
                645                 650                 655

Gln Ala Leu Gln Val Phe Tyr Ile Lys Ser Ile Glu Pro Gln Lys Val
            660                 665                 670

Ser Thr Leu Gly Lys Ser Asn Val Ile Val Thr Gly Ala Asn Phe Thr
        675                 680                 685

Arg Ala Ser Asn Ile Thr Met Ile Leu Lys Gly Thr Ser Thr Cys Asp
    690                 695                 700

Lys Asp Val Ile Gln Val Ser His Val Leu Asn Asp Thr His Met Lys
705                 710                 715                 720

Phe Ser Leu Pro Ser Ser Arg Lys Glu Met Lys Asp Val Cys Ile Gln
                725                 730                 735

Phe Asp Gly Gly Asn Cys Ser Ser Val Gly Ser Leu Ser Tyr Ile Ala
            740                 745                 750

Leu Pro His Cys Ser Leu Ile Phe Pro Ala Thr Thr Trp Ile Ser Gly
        755                 760                 765

Gly Gln Asn Ile Thr Met Met Gly Arg Asn Phe Asp Val Ile Asp Asn
    770                 775                 780

Leu Ile Ile Ser His Glu Leu Lys Gly Asn Ile Asn Val Ser Glu Tyr
785                 790                 795                 800

Cys Val Ala Thr Tyr Cys Gly Phe Leu Ala Pro Ser Leu Lys Ser Ser
                805                 810                 815

Lys Val Arg Thr Asn Val Thr Val Lys Leu Arg Val Gln Asp Thr Tyr
```

820 825 830

Leu Asp Cys Gly Thr Leu Gln Tyr Arg Glu Asp Pro Arg Phe Thr Gly
835 840 845

Tyr Arg Val Glu Ser Glu Val Asp Thr Glu Leu Glu Val Lys Ile Gln
850 855 860

Lys Glu Asn Asp Asn Phe Asn Ile Ser Lys Lys Asp Ile Glu Ile Thr
865 870 875 880

Leu Phe His Gly Glu Asn Gly Gln Leu Asn Cys Ser Phe Glu Asn Ile
885 890 895

Thr Arg Asn Gln Asp Leu Thr Thr Ile Leu Cys Lys Ile Lys Gly Ile
900 905 910

Lys Thr Ala Ser Thr Ile Ala Asn Ser Ser Lys Lys Val Arg Val Lys
915 920 925

Leu Gly Asn Leu Glu Leu Tyr Val Glu Gln Glu Ser Val Pro Ser Thr
930 935 940

Trp Tyr Phe Leu Ile Val Leu Pro Val Leu Leu Val Ile Val Ile Phe
945 950 955 960

Ala Ala Val Gly Val Thr Arg His Lys Ser Lys Glu Leu Ser Arg Lys
965 970 975

Gln Ser Gln Gln Leu Glu Leu Leu Glu Ser Glu Leu Arg Lys Glu Ile
980 985 990

Arg Asp Gly Phe Ala Glu Leu Gln Met Asp Lys Leu Asp Val Val Asp
995 1000 1005

Ser Phe Gly Thr Val Pro Phe Leu Asp Tyr Lys His Phe Ala Leu Arg
1010 1015 1020

Thr Phe Phe Pro Glu Ser Gly Gly Phe Thr His Ile Phe Thr Glu Asp
1025 1030 1035 1040

Met His Asn Arg Asp Ala Asn Asp Lys Asn Glu Ser Leu Thr Ala Leu
1045 1050 1055

Asp Ala Leu Ile Cys Asn Lys Ser Phe Leu Val Thr Val Ile His Thr
1060 1065 1070

Leu Glu Lys Gln Lys Asn Phe Ser Val Lys Asp Arg Cys Leu Phe Ala
1075 1080 1085

Ser Phe Leu Thr Ile Ala Leu Gln Thr Lys Leu Val Tyr Leu Thr Ser
1090 1095 1100

```
Ile Leu Glu Val Leu Thr Arg Asp Leu Met Glu Gln Cys Ser Asn Met
1105                1110                1115                1120

Gln Pro Lys Leu Met Leu Arg Arg Thr Glu Ser Val Val Glu Lys Leu
                1125                1130                1135

Leu Thr Asn Trp Met Ser Val Cys Leu Ser Gly Phe Leu Arg Glu Thr
            1140                1145                1150

Val Gly Glu Pro Phe Tyr Leu Leu Val Thr Thr Leu Asn Gln Lys Ile
        1155                1160                1165

Asn Lys Gly Pro Val Asp Val Ile Thr Cys Lys Ala Leu Tyr Thr Leu
    1170                1175                1180

Asn Glu Asp Trp Leu Leu Trp Gln Val Pro Glu Phe Ser Thr Val Ala
1185                1190                1195                1200

Leu Asn Val Val Phe Glu Lys Ile Pro Glu Asn Glu Ser Ala Asp Val
                1205                1210                1215

Cys Arg Asn Ile Ser Val Asn Val Leu Asp Cys Asp Thr Ile Gly Gln
            1220                1225                1230

Ala Lys Glu Lys Ile Phe Gln Ala Phe Leu Ser Lys Asn Gly Ser Pro
        1235                1240                1245

Tyr Gly Leu Gln Leu Asn Glu Ile Gly Leu Glu Leu Gln Met Gly Thr
    1250                1255                1260

Arg Gln Lys Glu Leu Leu Asp Ile Asp Ser Ser Ser Val Ile Leu Glu
1265                1270                1275                1280

Asp Gly Ile Thr Lys Leu Asn Thr Ile Gly His Tyr Glu Ile Ser Asn
                1285                1290                1295

Gly Ser Thr Ile Lys Val Phe Lys Lys Ile Ala Asn Phe Thr Ser Asp
            1300                1305                1310

Val Glu Tyr Ser Asp Asp His Cys His Leu Ile Leu Pro Asp Ser Glu
        1315                1320                1325

Ala Phe Gln Asp Val Gln Gly Lys Arg His Arg Gly Lys His Lys Phe
    1330                1335                1340

Lys Val Lys Glu Met Tyr Leu Thr Lys Leu Leu Ser Thr Lys Val Ala
1345                1350                1355                1360

Ile His Ser Val Leu Glu Lys Leu Phe Arg Ser Ile Trp Ser Leu Pro
                1365                1370                1375

Asn Ser Arg Ala Pro Phe Ala Ile Lys Tyr Phe Phe Asp Phe Leu Asp
            1380                1385                1390
```

```
Ala Gln Ala Glu Asn Lys Lys Ile Thr Asp Pro Asp Val Val His Ile
        1395                1400                1405

Trp Lys Thr Asn Ser Leu Pro Leu Arg Phe Trp Val Asn Ile Leu Lys
    1410                1415                1420

Asn Pro Gln Phe Val Phe Asp Ile Lys Lys Thr Pro His Ile Asp Gly
1425                1430                1435                1440

Cys Leu Ser Val Ile Ala Gln Ala Phe Met Asp Ala Phe Ser Leu Thr
                1445                1450                1455

Glu Gln Gln Leu Gly Lys Glu Ala Pro Thr Asn Lys Leu Leu Tyr Ala
            1460                1465                1470

Lys Asp Ile Pro Thr Tyr Lys Glu Glu Val Lys Ser Tyr Tyr Lys Ala
        1475                1480                1485

Ile Arg Asp Leu Pro Pro Leu Ser Ser Ser Glu Met Glu Glu Phe Leu
    1490                1495                1500

Thr Gln Glu Ser Lys Lys His Glu Asn Glu Phe Asn Glu Glu Val Ala
1505                1510                1515                1520

Leu Thr Glu Ile Tyr Lys Tyr Ile Val Lys Tyr Phe Asp Glu Ile Leu
                1525                1530                1535

Asn Lys Leu Glu Arg Glu Arg Gly Leu Glu Glu Ala Gln Lys Gln Leu
            1540                1545                1550

Leu His Val Lys Val Leu Phe Asp Glu Lys Lys Lys Cys Lys Trp Met
        1555                1560                1565
```

INTERNATIONAL SEARCH REPORT

Interna pplication No
PCT/US 00/24556

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/395 A61K38/17 A61P35/00 A61P37/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 32622 A (ZYMOGENETICS INC) 1 July 1999 (1999-07-01) cited in the application page 39, line 25 -page 40, line 17 claims 1-8,28,30	1
X	WO 99 29729 A (TAKASHIMA SEIJI ;KLAGSBRUN MICHAEL (US); MIAO HUA QUAN (US); SOKER) 17 June 1999 (1999-06-17) page 15, line 11 -page 16, line 23 page 22, line 4-7; claims 1,3,4	1
X	WO 99 04263 A (GINTY DAVID D ;KOLODKIN ALEX L (US); UNIV JOHNS HOPKINS (US)) 28 January 1999 (1999-01-28) page 9, line 17 -page 10, paragraph 2	2

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 January 2001	13/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Muller-Thomalla, K

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intern Application No
PCT/US 00/24556

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 21997 A (JOHNSON RICHARD S ;SPRIGGS MELANIE KAY (US); IMMUNEX CORP (US); CO) 6 May 1999 (1999-05-06) cited in the application page 3, line 13 -page 4, line 11	2
A	COMEAU M R ET AL: "A poxvirus-encoded semaphorin induces cytokine production from monocytes and binds to a novel cellular semaphorin receptor, VESPR" IMMUNITY,US,CELL PRESS, vol. 8, 1 April 1998 (1998-04-01), pages 473-482, XP002094205 ISSN: 1074-7613 the whole document	1,2
A	KOLODKIN A L ET AL: "Neuropilin is a semaphorin III receptor" CELL,CELL PRESS, CAMBRIDGE, NA,US, vol. 90, no. 4, 22 August 1997 (1997-08-22), pages 753-762, XP002084496 ISSN: 0092-8674 the whole document	1,2
A	SPRIGGS MELANIE K: "Shared resources between the neural and immune systems: Semaphorins join the ranks." CURRENT OPINION IN IMMUNOLOGY, vol. 11, no. 4, August 1999 (1999-08), pages 387-391, XP002158431 ISSN: 0952-7915 the whole document	1,2

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern pplication No
PCT/US 00/24556

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9932622 A	01-07-1999	AU 2000299 A	12-07-1999
WO 9929729 A	17-06-1999	AU 1633999 A	28-06-1999
		AU 1810499 A	28-06-1999
		AU 1810899 A	28-06-1999
		AU 1906099 A	28-06-1999
		EP 1037981 A	27-09-2000
		EP 1037925 A	27-09-2000
		EP 1037994 A	27-09-2000
		WO 9929861 A	17-06-1999
		WO 9930157 A	17-06-1999
		WO 9929858 A	17-06-1999
WO 9904263 A	28-01-1999	AU 8405398 A	10-02-1999
WO 9921997 A	06-05-1999	AU 1204799 A	17-05-1999
		EP 1027436 A	16-08-2000